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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Jesus Prieto VALTUENA, et al
Serial No.: 09/674,445 Group No.: 1614
Filed: November 1, 2000 Examiner.: Jegatheesan Seharaseyon
For: UTILIZATION OF INTERFERON ALPHA 5 IN THE TREATMENT OF VIRAL HEPATOPATHIES

Attorney Docket No.: U 013039-2

Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 CFR 1.132

I, Jesús Prieto, hereby declare:

1. I am one of the co-inventors of the subject matter described and claimed in the above application. I make this declaration in support of the application. A copy of my curriculum vitae is annexed hereto as Exhibit 1.

2. I have recently co-authored a paper (sent for publication) that describes experimentation that compares the specific antiviral action in liver of IFN alpha 5 with another well known interferon subtype (IFN alpha 2) broadly used in antiviral therapy of HCV. A copy of the paper is annexed hereto as Exhibit 1. I conducted or supervised the experimentation described in the paper and can attest that the reported results are accurate.

3. The results of the experimentation described in the paper show that the antiviral action attributed to IFN alpha 5, measured as cell signaling and antiviral gene induction, was more efficient and intense than when IFN alpha 2 was used. Although only HCV therapy is exemplified in the paper, the role of liver natural defense of IFN alpha 5 would not be expected to differ in other liver diseases of viral origin.

4. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and

further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity or the application of any patent issued thereon.

Date: 23.09.03

JESUS PRIETO VALTUEÑA

Name:

BIOGRAPHICAL SKETCH

NAME **PRIETO, JESUS** BIRTH: Oviedo (Spain) April,6 1944 [DNI:10486228]	POSITION TITLE **PROFESSOR**

EDUCATION AND TRAINING

INSTITUTION AND LOCATION	DEGREE	YEAR	FIELD OF STUDY
University of Valladolid (Spain)	M.D.	1967	Medicine
University of Valladolid	Ph. D.	1969	Medicine (Hepatology)
University Hospital of Valladolid	Board Certifications in Gastroenterology and Internal Medicine	1969 1970	Gastroenterology/Int. Medicine
Royal Free Hospital. London (Prof. Sheila Sherlock)	Post Doctoral Studies and Clinical Assistant	1972-73	Hepatology

PROFESSIONAL EXPERIENCE

INSTITUTION AND LOCATION	TITLE	YEAR	FIELD
University Hospital of Valladolid (Spain)	Assitant Professor and Clinical Assistant	1970-72 1974-75	Int.Medicine/ Gastro/Hepatol
University Hospital of Valladolid	Associate Professor	1976	Medicine
University of Oviedo (Spain)	Professor of Medicine	1976-77	Medicine
University of Santiago de Compostela and General Hospital of Galicia (Spain)	Professor of Medicine and Chairman Department of Medicine	1977-79	Int. Medicine/Gastro/Hepatol.
University of Navarra and Clinica Universitaria de Navarra (Spain)	Professor of Medicine co-Chairman Department of Medicine and Liver Unit	1979-85	Int. Medicine/Gastro/Hepatol
University of Navarra and Clinica Universitaria de Navarra	Professor of Medicine Chairman Department of Medicine and Liver Unit	1985-1996	Int. Medicine/Gastro/Hepatol
University of Navarra and Clinica Universitaria de Navarra	Professor of Medicine Chairman Department of Medicine and Chief Division of Hepatology and Gene Therapy	1997-	Int. Medicine/Gastro/Hepatol

AWARDS AND HONORS

- President of the Spanish Association for the Study of the Liver (2001-)
- Vice-President of the Spanish Association for the Study of the Liver (1985-89)
- President of the Society of Internal Medicine of Navarra, Aragon and Basque Country (1993-94)
- Member of the Scientific Committee of the European Association for the Study of the Liver (1989-92)
- Doctor Honoris Causa . Faculty of Medicine. University of Porto (Portugal)
- Founder of the Spanish Society of Gene Therapy (2000)
- Member of the Scientific Board of ANRS (Agencie Nationale Francaise pour la Recherche sur le SIDA et l'Hepatite C- National French Agency for Investigation on AIDS and Hepatitis C) 2000-
- Expert of INSERM (Institute National Francais pour la Santé et la Recherche Medicale; National French Institute for Health and Medical Research) 2000-
- Member of the Committe of Experts of the Spanish Ministry of Health for evaluation of Interferon therapy. 1998
- Expert of the Spanish Ministry of Health for evaluation of new drugs (Agencia Española del Medicamento) 2000-
- Member or ex- member of the Editorial Committee of Hepatology Research, Journal of Hepatology, Alimentary Tract Pharmacology and Therapeutics, Revista Clinica Española, Medicina Clinica, Hepatologia y Gastroenterologia, Revista Española Enfermedades del Aparato Digestivo.
- Award "Candida Medrano de Merlo" for the work on "Gene Therapy of Liver Cancer"
- Awarded spanish patent for the use of Interferon alpha 5 for the treatment of liver diseases of viral and neoplastic origin. P 980 1003 (BOE 16/Aug/2000) (pending the european patent)
- Several papers were commented in different issues of *Year Book of Medicine* and deserved editorials in New England Journal of Medicine, Gastroenterology, Hepatology, Gut and other journals.
- Invited speaker in international symposia and meetings of different national Societies of Hepatology and Gastroenterology such as: Meeting of the French Association for the Study of the Liver (Paris, 1993), European Association for the Study of the Liver (Naples, 1999), Spanish Society of Gastroenterology (Madrid 1997), Asian Symposium on Liver Diseases (Beijing 1999), European Gastroenterological Week (Brussels, 2000), Italian Association for the Study of the Liver (Rome,

2001), American Association for the Study of the Liver (Single Topic Conference, Airlie, Virginia, 2001), British Association for the Study of the Liver (London 2001), Polish Association for the Study of the Liver (Mikolajki, Poland, 2001).

PUBLICATIONS

1. "Die Elimination von Bromosulphalein (BSP): Mathematische Untersuchung des Verhaltens dieser Substanz bei intravenoser Anwendung in Einer Einzeldosis". J. Prieto Valtueña, T. Calvo del Olmo, S. de Castro del Pozo. **ACTA HEPATOGASTROENTEROLOGICA** 1972; 19: 352.
2. ."Serum Ferritin in Patients with Iron Overload and with Acute and Cronic Liver Disease". J. Prieto, M. Barry and S. Sherlock. **GASTROENTEROLOGY** 1975; 68: 525.
3. "Serum Ferritin Assay and Iron Status ion Chronic Renal Failure and Haemodialysis". S. Hussein, J. Prieto, M. O'Shea, A.V. Hoffbrand, R.A. Baillod, J.F. Moorhead. **BRITISH MEDICAL JOURNAL** 1975; 1: 546.
4. "Immune complexes in epidemic (type A) hepatitis. Detection by three methods using laser nephelometry". M. Serrano, J. Prieto, J.M. Esteban and C.D. Crisci. **ALLERGOLOGY ET IMMUNOPATHOLOGY** 1981; 9,5: 433-440.
5. "Mediation of a receptor mechanism in the uptake of iron from transferrin by the hepatocyte". R.M. Nunes, J.M. Prieto and B.J. Potter. **PROTIDES OF THE BIOLOGICAL FLUIDS 29 th COLLOQUIUM 1981**. Edited by H. Peeters. Pergamon Press. Oxford and New York, pag. 455-458, 1982.
6. "Serum Antibodies Against Porphyric Hepatocytes in Patients with porphyria cutanea tarda and liver disease". E. Baravalle and J. Prieto. **GASTROENTEROLOGY** 1983; 84: 1483-1491.
7. "Intracerebroventricular infusion of sodium chloride-rich artificial cerebrospinal fluid in rats induces natriuresis and releases an inhibitor of prostaglandin synthesis". J. Díez, I. Colina, F. Guarner, J. Quiroga, J. Corzo, A. Purroy and J. Prieto. **CLINICAL SCIENCE** 1984; 66: 621-624.
8. "Cytoprotective effect of prostaglandins on isolated rat liver cells". F. Guarner, M. Fremont-Smith and J. Prieto. **LIVER** 1985; 5: 35-39.

9. "Increased Synthesis of Systemic Prostacyclin in Cirrhotic Patients". F. Guarner, C. Guarner, J. Prieto, I. Colina. J. Quiroga, J. Casas, R. Freixa, J. Roselló, E. Gelpi and J. Balanzó. **GASTROENTEROLOGY** 1986; 90: 687-694.
10. "Renal prostaglandins in cirrhosis of the liver". C Guarner, I. Colina, F. Guarner, J. Corzo, J. Prieto and F. Vilardell. **CLINICAL SCIENCE** 1986; 70: 477-484.
11. "Effect of Spironolactone on Renal Prostaglandin Excretion in Patients with Liver Cirrhosis and Ascites". J.F. Medina, J. Prieto, F. Guarner, J. Quiroga and A. Milazzo. **JOURNAL OF HEPATOLOGY** 1986; 3: 206-211.
12. "Interleukins in chronic active hepatitis B: Relationship with viral markers". Maria Pilar Civeira, Jesús Prieto, Susana Morte, Marta Riñón and Manuel Serrano. **JOURNAL OF HEPATOLOGY** 1987; 5: 37-44.
13. "Monocyte Function in Chronic Non-A, Non B Hepatitis: Relationship with the Activity of Liver Disease". A. Castilla, M. Serrano, S. Morte, M.L. Subirá, M.P. Civeira and J. Prieto. **VIRAL HEPATITIS AND LIVER DISEASE** 1988; Alan R, Liss, Inc., pág. 568-571.
14. "Gamma-Interferon Production by Peripheral Mononuclear Cells in Patients With Chronic Non-A, Non-B Hepatitis". M. Serrano, S. Morte, A. Castilla, M.P. Civeira and J. Prieto. **VIRAL HEPATITIS AND LIVER DISEASE** 1988; Alan R, Liss, Inc., pag. 572-575.
15. "Opioid peptides modulate the organization of vimentin filaments, the phagocytic activity and the expression of surface molecules in monocytes". J. Prieto, M.L. Subirá, A. Castilla, J.L. Arroyo, M. Serrano. **SCANDINAVIAN JOURNAL OF IMMUNOLOGY** 1989; 29: 391-398.
16. "Cytoskeletal Organization and Functional Changes in Monocytes from Patients with Chronic Hepatitis B: Relationship with Viral Replication". J. Prieto, A. Castilla, M.L. Subirá, M. Serrano, S. Morte and M.P. Civeira. **HEPATOLOGY** 1989; 9 (5): 720-725.
17. "Naloxone-Reversible Monocyte Dysfunction in Patients with Chronic Fatigue Syndrome". J. Prieto, M.L. Subirá, A. Castilla and M. Serrano. **SCANDINAVIAN JOURNAL OF IMMUNOLOGY** 1989; 30: 13-20.

18. "Systemic and Regional Hemodynamics in Patients With Liver Cirrhosis and Ascites With and Without Functional Renal Failure". J. Fernández-Seara, J. Prieto, J. Quiroga, JM. Zozaya, MA. Cobos, JL. Rodríguez-Eire, A. García-Plaza and J. Leal. **GASTROENTEROLOGY** 1989; 97: 1304-1312.
19. "Monocyte disorder causing cellular immunodeficiency: a family study". J. Prieto, M.L. Subirá, A. Castilla, M.P. Civeira and M. Serrano. **CLINICAL EXPERIMENTAL IMMUNOLOGY** 1990; 79: 1-6.
20. "Transforming Growth Factors Betal and Alpha in Chronic Liver Disease. Effects of Interferon Alfa Therapy". A. Castilla, J. Prieto and N. Fausto. **NEW ENGLAND JOURNAL OF MEDICINE** 1991; 324 (14): 933-940.
21. "Inhibitors of the lipoxygenase arachidonic acid pathway impair glycocholate efflux in isolated rat hepatocytes". J. Quiroga, J.L. Rodríguez-Sanromán, F. Guarner, C. Rodríguez Ortigosa, J.M. Aréjola and J. Prieto. **JOURNAL OF HEPATOLOGY** 1991; 12: 302-311.
22. "Enhanced responsiveness to CNS-induced natriuresis in anesthetized nonascitic cirrhotic rats". I. Colina, J. Quiroga, F. Guarner, A. Purroy and J. Prieto. **AMERICAN JOURNAL OF PHYSIOLOGY** 1991; 260: G972-G976.
23. "Liver changes in patients with hyperthyroidism". J. Sola, F.J. Pardo-Mindán, J. Zozaya, J. Quiroga, B. Sangro and J. Prieto. **LIVER** 1991; 11: 193-197.
24. "Abnormal Sympathetic and Renal Response to Sodium Restriction in Compensated Cirrhosis". M.A. Simón, J. Diez and J. Prieto. **GASTROENTEROLOGY** 1991; 101: 1354-1360.
25. "Detection of hepatitis C virus antibodies with new recombinant antigens: assessment in chronic liver diseases". J.I. Riezu-Boj, D. Parker, M.P. Civeira, D. Phippard, T.P. Corbishley, J. Camps, A. Castilla and J. Prieto. **JOURNAL OF HEPATOLOGY** 1992; 15: 309-313.
26. "Hepatitis B and C viral infections in patients with hepatocellular carcinoma". J. Ruiz, B. Sangro, JI. Cuende, O. Beloqui, JI. Riezu-Boj, JI. Herrero and J. Prieto. **HEPATOLOGY** 1992; Vol. 16, No. 3: 637-641.

27. "Replication of hepatitis C virus in peripheral blood mononuclear cells: effect of alpha-interferon therapy". C. Qian, J. Camps, MD. Maluenda, MP. Civeira and J. Prieto. **JOURNAL OF HEPATOLOGY** 1992; 16: 380-383.
28. "Splenic embolization prior to myelosuppressive treatment in hepatocarcinoma and active chronic hepatitis". J.I. Bilbao, B. Sangro, J.M. Longo, J.M. Zozaya, A. Fernández-Virgós, J.D. Aquerreta, O. Beloqui, J. Prieto. **EUROPEAN JOURNAL OF RADIOLOGY** 1992; 15: 211-214.
29. "Arteriovenous Shunting, Hemodynamic Changes, and Renal, Sodium Retention in Liver Cirrhosis". C.M. Fernández-Rodriguez, J. Prieto, J.M. Zozaya, J. Quiroga and R. Guitián. **GASTROENTEROLOGY** 1993; 104: 1139-1145.
30. "Detection of anti-hepatitis C Virus Antibodies by ELISA using synthetic peptides". C. Berasain, M. García-Granero, J.I. Riezu-Boj, M.P. Civeira, J. Prieto and F. Borrás-Cuesta. **JOURNAL OF HEPATOLOGY** 1993; 18: 80-84.
31. "Partial Splenic Embolization for the Treatment of Hypersplenism in Liver Cirrhosis". B. Sangro, I. Bilbao, I. Herrero, C. Corella, J. Longo, O. Beloqui, J. Ruiz, JM. Zozaya, J. Quiroga, J. Prieto. **HEPATOLOGY** 1993; 18 (2): 309-314.
32. "Histological outcome of chronic hepatitis C treated with a 12-month course of lymphoblastoid alfa interferon". E. de Alava, J. Camps, J. Pardo-Mindán, M. García-Granero, M. Muñoz, J. Sola, MP. Civeira, F. Contreras, JJ. Vázquez, A. Castilla, J. Prieto. **LIVER** 1993; 13: 73-79.
33. "Abnormal Expression of Anion Exchanger Genes in Primary Biliary Cirrhosis". J. Prieto, C. Qian, N. García, J. Diez, J.F. Medina. **GASTROENTEROLOGY** 1993; 105: 572-578.
34. "Randomised trial of lymphoblastoid alfa-interferon in chronic hepatitis C. Effects on inflammation, fibrogenesis and viremia". J. Camps, A. Castilla, J. Ruiz, MP. Civeira and J. Prieto. **JOURNAL OF HEPATOLOGY** 1993; 17: 390-396.
35. "Hepatitis B virus occult infection in subjects with persistent isolated anti-HBc reactivity". A. Sánchez-Quijano, J.I. Jauregui, M. Leal, J.A. Pineda, A. Castilla, M.A. Abad, M.P. Civeira, F. García de Pesquera, J. Prieto and E. Lissen. **JOURNAL OF HEPATOLOGY** 1993; 17: 288-293.

36. "Prediction of the response of chronic hepatitis C to interferon alfa: a statistical analysis of pretreatment variables". J. Camps, S. Crisóstomo, M. García-Granero, J.I. Riezu-Boj, M.P. Civeira, J. Prieto. **GUT** 1993; 34: 1714-1717.
37. "Hepatic and Extrahepatic HCV RNA Strands in Chronic Hepatitis C: Different Patterns of Response to Interferon Treatment". B. Gil, Ch. Qian, J.I. Riezu-Boj, M.P. Civeira, J. Prieto. **HEPATOLOGY** 1993; 18 (5): 1050-1054.
38. "Ribavirin in the treatment of chronic hepatitis C unresponsive to alfa interferon". J. Camps, N. García, J.I. Riezu-Boj, M.P. Civeira, J. Prieto. **JOURNAL OF HEPATOLOGY** 1993; 19: 408-412.
39. "Transjugular Intrahepatic Portosystemic Shunts Using the Wallstent Prosthesis: A Follow-Up Study". H. Rousseau, J.P. Vinel, J.I. Bilbao, J.M. Longo, P. Maquin, J.M. Zozaya, L. García-Villarreal, B. Cousted, N. Railhac, J.J. Railhac, J. Alvarez-Cienfuegos, J. Prieto, F. Joffre, J.P. Pascal. **CARDIOVASCULAR AND INTERVENTIONAL RADIOLOGY** 1994; 17: 7-11.
40. "Immunohistochemical Detection of Chloride/bicarbonate Anion Exchangers in Human Liver". E. Martinez-Ansó, J.E. Castillo, J. Díez, J.F. Medina, J. Prieto. **HEPATOLOGY** 1994; 19 (6): 1400-1406.
41. "Prediction of sustained remission of chronic hepatitis C after a 12-month course of alfa interferon". J. Camps, M. García-Granero, J.I. Riezu-Boj, E. Larrea, E. de Alava, M.P. Civeira, A. Castilla, J. Prieto. **JOURNAL OF HEPATOLOGY** 1994; 21: 4-11.
42. "Atrial natriuretic factor in cirrhosis: relationship to renal function and hemodynamic changes". C. Fernández-Rodriguez, J. Prieto, J. Quiroga, J.M. Zozaya, A. Andrade, D. Rodriguez-Martinez. **JOURNAL OF HEPATOLOGY** 1994; 21: 211-216.
43. "Overcoming class II-linked non-responsiveness to hepatitis B vaccine". S. Hervás-Stubbs, C. Berasain, J.J. Golvano, J.J. Lasarte, I. Prieto, P. Sarobe, J. Prieto, F. Borrás-Cuesta. **VACCINE** 1994; 12 (10): 867-871.
44. "Plasma Levels of Substance P in Liver Cirrhosis: Relationship to the Activation of Vasopressor Systems and Urinary Sodium Excretion". C.M. Fernández-Rodriguez, J. Prieto, J. Quiroga, J.M. Zozaya, A. Andrade, M. Núñez, B. Sangro, J. Penas. **HEPATOLOGY** 1995; 21 (1): 35-40.

45. "Anion Exchanger Immunoreactivity in Human Salivary Glands in Health and Sjögren's Syndrome. J.J. Vázquez, M. Vázquez, M.A. Idoate, L. Montuenga, E. Martinez-Ansó, J.E. Castillo, N. García, J.F. Medina, J. Prieto. **AMERICAN JOURNAL OF PATHOLOGY** 1995; 146 (6): 1422-1432.
46. "Induction of Sensitivity to Ganciclovir in Human Hepatocellular Carcinoma Cells by Adenovirus-Mediated Gene Transfer of Herpes Simplex Virus Thymidine Kinase". Ch. Qian, R. Bilbao, O. Bruña, J. Prieto. **HEPATOLOGY** 1995; 22 (1): 118-123.
47. "Taurocholate-Stimulated Leukotriene C4 Biosynthesis and Leukotriene C4-Stimulated Choleresis in Isolated Rat Liver. C.M. Rodriguez-Ortigosa, I. Vesperinas, Ch. Qian, J. Quiroga, J.F. Medina, J. Prieto. **GASTROENTEROLOGY** 1995; 108: 1793-1801.
48. "Transjugular Intrahepatic Portal-systemic Shunt in the Treatment of Refractory Ascites: Effect on Clinical, Renal, Humoral, and Hemodynamic Parameters". J. Quiroga, B. Sangro, M. Núñez, I. Bilbao, J. Longo, L. García-Villarreal, J.M. Zozaya, M. Betes, J.I. Herrero, J. Prieto. **HEPATOLOGY** 1995; 21 (4): 986-994.
49. "The GCGGAA gene-regulatory motif of herpes simplex virus type-1 is also found in hepatitis C virus". J.L. Vizmanos, J.I. Jauregui, A. Gullón, C.J. González, M.P. Civeira, J. Prieto, M. García-Delgado. **GENE** 1995; 154: 131-132.
50. "Anticardiolipin Antibodies in Chronic Hepatitis C: Implication of Hepatitis C Virus as the Cause of the Antiphospholipid Syndrome". J. Prieto, J.R. Yuste, O. Beloqui, M.P. Civeira, J.I. Riezu, B. Aguirre, B. Sangro. **HEPATOLOGY** 1996; 23 (2): 199-204.
51. "Tumor Necrosis Factor α Gene Expression and the Response to Interferon in Chronic Hepatitis C". E. Larrea, N. García, Ch. Qian, M.P. Civeira, J. Prieto. **HEPATOLOGY** 1996; 23 (2): 210-217.
52. "Production of interleukin-2 in response to synthetic peptides from hepatitis C virus E1 protein in patients with chronic hepatitis C: relationship with the response to interferon treatment". P. Sarobe, J.I. Jauregui, J.J. Lasarte, N. García, M.P. Civeira, F. Borrás-Cuesta, J. Prieto. **JOURNAL OF HEPATOLOGY** 1996; 25 (1): 1-9.
53. "Epidemiological, clinical and therapeutic associations of hepatitis C types in western European patients". P. Simmonds, J. Mellor, A. Craxi, J.M. Sanchez-Tapias, A. Alberti, J. Prieto, M. Colombo, M.G. Rumi, O. Lo Iacano, S. Ampurdanes-Mingall, X. Forns-Bernhardt, L.

Chemello, M.P. Civeira, C. Frost, G. Dusheiko. **JOURNAL OF HEPATOLOGY** 1996; 24: 517-524.

54. "Molecular Cloning and Characterization of the Human Anion Exchanger 2 (SLC4A2) Gene". J.F. Medina, A. Acin, J. Prieto. **GENOMICS** 1997; 39: 74-85.
55. "Decreased Anion Exchanger-2 Immunoreactivity in the Liver of Patients With Primary Biliary Cirrhosis". J.F. Medina, E. Martinez-Ansó, J.J. Vázquez, J. Prieto. **HEPATOLOGY** 1997; 25 (1): 12-17.
56. "Low doses of insulin-like growth factor-I improve nitrogen retention and food efficiency in rats with early cirrhosis". A. Picardi, A. Costa de Oliveira, B. Muguerza, A. Tosar, J. Quiroga, I. Castilla-Cortázar, S. Santidrián, J. Prieto. **JOURNAL OF HEPATOLOGY** 1997; 26: 191-202.
57. "Induction of Cytotoxic T-Cell Response Against Hepatitis C Virus Structural Antigens Using a Defective Recombinant Adenovirus". O. Bruña-Romero, J.J. Lasarte, G. Wilkinson, K. Grace, B. Clarke, F. Borrás-Cuesta, J. Prieto. **HEPATOLOGY** 1997; 25 (2): 470-477.
58. "Gene Transfer and Therapy with Adenoviral Vector in Rats with Diethylnitrosamine-Induced Hepatocellular Carcinoma". C. Qian, M. Idoate, R. Bilbao, B. Sangro, O. Bruña, J. Vazquez, J. Prieto. **HUMAN GENE THERAPY** 1997; 8: 349-358.
59. "Partial splenic embolization in the treatment of thrombocytopenia following liver transplantation. J.I. Herrero, B. Sangro, J. Quiroga, J.I. Bilbao, J.R. Yuste, J. Longo, F. Pardo, J.J. Hernández, J. A.Cienfuegos, J. Prieto. **TRANSPLANTATION** 1997; 63 (3): 482-484.
60. "S-Adenosyl-l-Methionine Protects the Liver Against the Cholestatic, Cytotoxic, and Vasoactive Effects of Leukotriene D_4: A Study With Isolated and Perfused Rat Liver". R.N. Cincu, C.M. Rodriguez-Ortigosa, I. Vesperinas, J. Quiroga, J. Prieto. **HEPATOLOGY** 1997; 26 (2): 330-335.
61. "Viremia After One Month of Interferon Therapy Predicts Treatment Outcome in Chronic Hepatitis C". B. Gavier, M.A. Martínez-González, J.I. Riezu-Boj, J.J. Lasarte, N. García, M.P. Civeira, J. Prieto. **GASTROENTEROLOGY** 1997; 113: 1647-1653.
62. "Hepatoprotective effects of Insulin-like Growth Factor I in Rats with Carbon Tetrachloride-induced Cirrhosis". I. Castilla-Cortázar, M. García, B. Muguerza, J. Quiroga, R. Perez, S. Santidrian, J. Prieto. **GASTROENTEROLOGY** 1997; 113: 1682-1691.

63. "Impaired Intestinal Sugar Transport in Cirrhotic Rats: Correction by low doses of Insulin-like Growth Factor I". I. Castilla-Cortázar, J. Prieto, E. Urdaneta, M. Pascual, M. Nuñez, E. Zudaire, M. García, J. Quiroga, S. Santidrian. **GASTROENTEROLOGY** 1997; 113: 1180-1187.
64. "Therapeutic vaccination of woodchucks against chronic woodchuck hepatitis virus infection". S. Hervás-Stubbs, J.J. Lasarte, P. Sarobe, J. Prieto, J. Cullen, M. Roggendorf, F. Borrás-Cuesta. **JOURNAL OF HEPATOLOGY** 1997; 27: 726-737.
65. "Risk Factors for Recurrence of Hepatitis C After Liver Transplantation". J.I. Herrero, A. de la Peña, J. Quiroga, B. Sangro, N. García, I. Sola, J.A. Cienfuegos, M.P. Civeira, J. Prieto. **LIVER TRANSPLANTATION AND SURGERY** 1998; 4 (4): 265-270.
66. "Transmission of Hepatitis C Virus Infection to Tree Shrews". Z.C. Xie, J.I. Riezu-Boj, J.J. Lasarte, J. Guillen, J.H. Su, M.P. Civeira, J. Prieto. **VIROLOGY** 1998; 244: 513-520.
67. "Superoxide Dismutase in Patients with Chronic Hepatitis C Virus Infection". E. Larrea, O. Beloqui, M.A. Muñoz-Navas, M.P. Civeira, J. Prieto. **FREE RADICAL BIOLOGY & MEDICINE** 1998; 24 (7-8): 1235-1241.
68. "Circulating adrenomedullin in cirrhosis: relationship to hyperdynamic circulation". C.M. Fernández-Rodriguez, I.R. Prada, J. Prieto, L.M. Montuenga, T. Elssasser, J. Quiroga, M. Moreiras, A. Andrade, F. Cuttitta. **JOURNAL OF HEPATOLOGY** 1998; 98: 250-256.
69. "Cellular Immunity to Hepatitis C Virus Core Protein and the Response to Interferon in Patients With Chronic Hepatitis C". J.J. Lasarte, M. García-Granero, A. López, N. Casares, N. García, M.P. Civeira, F. Borrás-Cuesta, J. Prieto. **HEPATOLOGY** 1998; 28 (3): 815-822.
70. "Prognosis of hepatocellular carcinoma in relation to treatment: A multivariate analysis of 178 patients from a single European institution". B. Sangro, M. Herráiz, M.A. Martinez-González, I. Bilbao, I. Herrero, O. Beloqui, M. Betes, A. de la Peña, J. A.Cienfuegos, J. Quiroga, J. Prieto. **SURGERY** 1998; 124 (3): 575-583.
71. "Transformed but not normal hepatocytes express UCP2". M.V. Carretero, L. Torres, M.U. Latasa, E. R. García-Trevijano, J. Prieto, J.M. Mato, M.A. Avila. **FEBS** Letters 1998; 439: 55-58.

72. "Antitumor effect of allogenic fibroblasts engineered to express Fas ligand (FasL)". M. Drozdzik, C. Qian, J.J. Lasarte, R. Bilbao, J. Prieto. **GENE THERAPY** 1998; 5: 1622-1630.
73. "Gene therapy of viral hepatitis and hepatocellular carcinoma". J. Ruiz, C. Qian, M. Drozdzik, J. Prieto. **JOURNAL OF VIRAL HEPATITIS** 1999; 6: 17-34.
74. "Immunogenicity of variable regions of hepatitis C virus proteins. Selection and modification of peptide epitopes to assess hepatitis C virus genotypes by ELISA". M. Rodríguez-López, J.I. Riezu-Boj, M. Ruiz, C. Berasain, M.P. Civeira, J. Prieto, F. Borrás-Cuesta. **JOURNAL OF GENERAL VIROLOGY** 1999; 80: 727-738.
75. "Effect of insulin-like growth factor I on in vivo intestinal absorption of D-galactose in cirrhotic rats". I. Castilla-Cortázar, A. Picardi, A. Tosar, J. Ainzúa, E. Urdaneta, M. García, M. Pascual, J. Quiroga, J. Prieto. **AMERICAN JOURNAL PHYSIOLOGY** 276 (Gastrointestinal liver physiology) 39 1999: G37-G42.
76. "Assessment of Biliary Bicarbonate Secretion in Humans by Positron Emission Tomography". J.Prieto, N. García, J.M. Martí-Climent, I. Peñuelas, J.A. Richter and J.F Medina. **GASTROENTEROLOGY** 1999; 117:167-172.
77. "Different doses of Adenoviral Vector Expressing IL-12 Enhance or Depress the Inmmune Response to a Coadministered Antigen: the Role of Nitic Oxide". J.J. Lasarte, F.J. Corrales, N. Casares, A. López-Díaz de Cerio, C. Quian, X. Xie, F. Borrás-Cuesta, J. Prieto. **THE JOURNAL OF IMMUNOLOGY** 1999; 162: 5270-5277.
78. "Interferon Alfa Subtypes and Levels of Type I Interferons in the Liver and Peripheral Mononuclear Cells in Patients with Chronic Hepatitis C and Controls". Yurdana Castelruiz, Esther Larrea, Patricia Boya, María-pilar Civeira , Jesús Prieto. **HEPATOLOGY** 1999;29:1900-1904
79. "Intratumoral injection of bone-marrow derived dendritic cells engineered to produce interleukin- 12 induces complete regression of established murine transplantable colon adenocarcinomas". I. Melero, M.Duarte, J. Ruiz, B. Sangro, JC Galofré, G. Mazzolini, M Bustos, C. Qian, J. Prieto. **GENE THERAPY** 1999; 6: 1779-1784.
80. "Conversion of Liver Transplant Recipients on Cyclosporine With Renal Impairment to Mycophenolate Mofetil". J.I. Herrero, J. Quiroga, B. Sangro, M. Girala, N. Gómez-Manero, F. Pardo, J.

Alvárez-Cienfuegos, J. Prieto. **LIVER TRANSPLANTATION AND SURGERY** 1999; Vol. 5 (5): 414-420.

81. "Regression of colon cancer and induction of antitumor immunity by intratumoral injection of adenovirus expressing interleukin-12". G. Mazzolini, C. Qian, X. Xie, Y. Sun, J.J. Lasarte, M. Drozdzik, J. Prieto. **CANCER GENE THERAPY** 1999; 6 (6) 514-522.
82. "Antioxidant status and glutathione metabolism in peripheral blood mononuclear cells from patients with chronic hepatitis C". P. Boya, A. de la Peña, O. Beloqui, E. Larrea, M. Conchillo, Y. Castelruiz, MP. Civeira, J. Prieto. **JOURNAL OF HEPATOLOGY** 1999; 31: 808-814.
83. "Early predictors of response to treatment in patients with chronic hepatitis C". M.P. Civeira, J. Prieto. **JOURNAL OF HEPATOLOGY** 1999; 31 (1): 237-243.
84. "Gene Transfer to Liver Cancer Cells of B7-1 Plus Interleukin 12 Changes Immunoeffector Mechanisms and Supresses Helper T Cell Type 1 Cytokine Production Induced by Interleukin 12 Alone". Y. Sun, C. Qian, D. Peng, J. Prieto. **HUMAN GENE THERAPY** 2000; 11: 127-138.
85. "Adenoviral Gene Transfer of Interleukin 12 into Tumors Synergizes with Adoptive T Cell Therapy Both at the Induction and Effector Level". G. Mazzolini, C. Qian, I. Narvaiza, M. Barajas, F. Borrás-Cuesta, X. Xie, M. Duarte, I. Melero, J. Prieto. **HUMAN GENE THERAPY** 2000; 11: 113-125.
86. "Insulin-like Growth Factor-I Reverts Testicular Atrophy in Rats With Advanced Cirrhosis". I. Castilla-Cortazar, M. García, J. Quiroga, N. Diez, F. Diez-Caballero, A. Calvo, M. Diaz, J. Prieto. **HEPATOLOGY** 2000; 31: 592-600.
87. "Intratumoral Coinjection of Two Adenoviruses, One Encoding the Chemokine IFN-γ-Inducible Protein-10 and Another Encoding IL-12, Results in Marked Antitumoral Synergy". I. Narvaiza, G. Mazzolini, M. Barajas, M. Duarte, M. Zaratiegui, C. Qian, I. Melero, J. Prieto. **THE JOURNAL OF IMMUNOLOGY** 2000; 164: 3112-3122.
88. "Combined gene therapy with suicide gene and interleukin-12 is more efficient than therapy with one gene alone in a murine model of hepatocellular carcinoma". M. Drozdzik, C. Qian, X. Xie, D. Peng, R. Bilbao, G. Mazzolini, J. Prieto. **JOURNAL OF HEPATOLOGY** 2000; 32: 279-286.

89. "The potential of gene therapy in the treatment of hepatocellular carcinoma". C. Qian, M. Drozdzik, W.H. Caselmann, J. Prieto. **JOURNAL OF HEPATOLOGY** 2000; 32: 344-351.
90. "Tissue-Specific N-Terminal Isoforms from Overlapping Alternate Promoters of the Human AE2 Anion Exchanger Gene". J.F. Medina, J. Lecanda, A. Acín, P. Ciesielczyk, J. Prieto. **BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS** 2000; 267: 228-235.
91. "In situ localization of anion exchanger-2 in the human kidney". J.E. Castillo, E. Martínez-Ansó, R. Malumbres, E. De Alava, C. García, J.F. Medina, J. Prieto. **CELL TISSUE RES** 2000; 299: 281-287.
92. "Transduction of hepatocellular carcinoma (HCC) using recombinant adeno-associated virus (rAAV): in vitro and in vivo effects of genotoxic agents". D. Peng, C. Qian, Y. Sun, M.A. Barajas, J. Prieto. **JOURNAL OF HEPATOLOGY** 2000; 32: 975-985.
93. "Altered intestinal transport of amino acids in cirrhotic rats: the effect of insulin-like growth factor-I". M. Pascual, I. Castilla-Cortazar, E. Urdaneta, J. Quiroga, M. García, A. Picardi, J. Prieto. **AMERICAN JOURNAL PHYSIOLOGY** (Gastrointestinal liver phisiology) 2000; 279: G319-G324.
94. "Transduction efficacy, antitumoral effect, and toxicity of adenovirus-mediated herpes simplex virus thymidine kinase/ganciclovir therapy of hepatocellular carcinoma: the woodchuck animal model". R. Bilbao, R. Gerolami, MP. Bralet, C. Qian, PL. Tran, B. Tennant, J. Prieto, C. Brechot. **CANCER GENE THERAPY** 2000; 7: 657-662.
95. "Liver damage using suicide genes: a model for oval cell activation". M. Bustos, B. Sangro, P. Alzuguren, AG. Gil, J. Ruiz, N. Beraza, C. Qian, A. García-Pardo, J. Prieto. **AMERICAN JOURNAL PATHOLOGY** 2000; 157: 549-559.
96. "Combination therapy with interferon-α plus *N*-acetyl cysteine for chronic hepatitis C: A placebo controlled double-blind multicentre study". P.R. Grant, A. Black, N. García, J. Prieto, J. A. Garson. **JOURNAL OF MEDICAL VIROLOGY** 2000, 61: 439-442.
97. "Pathological and virological findings in patients with persistent hypertransaminasaemia of unknown aetiology". C. Berasain, M. Betés, A. Panizo, J. Ruiz, J. I. Herrero, M-P Civeira, J. Prieto. **GUT** 2000, 47: 429-435.

98. "Hyperhomocysteinemia in Liver Transplant Recipients: Prevalence and Multivariate Analysis of Predisposing Factors". J.I. Herrero, J. Quiroga, B. Sangro, O. Beloqui, F. Pardo, J.A. Cienfuegos, J. Prieto. **LIVER TRANSPLANTATION** 2000; 6 (5): 614-618.
99. "In vivo gene transfer of CD40 ligand into colon cancer cells induces local production of cytokines and chemokines, tumor eradication and protective antitumor immunity". Y. Sun, D. Peng, J. Lecanda, V. Schmitz, M. Barajas, C. Qian, J. Prieto. **GENE THERAPY** 2000; 7: 1467-1476.
100. "Reduced mRNA abundance of the main enzymes involved in methionine metabolism in human liver cirrhosis and hepatocellular carcinoma". M.A. Avila, C. Berasin, L. Torres, A. Martín-Duce, F.J. Corrales, H. Yang, J. Prieto, S.C. Lu, J. Caballería, J. Rodés. J.M. Mato. **JOURNAL OF HEPATOLOGY** 2000; 33: 907-914.
101. "A blood-tumor barrier limits gene transfer to experimental liver cancer: the effect of vasoactive compounds". R. Bilbao, M. Bustos, P. Alzuguren, M.J. Pajares, M. Drozdzik, C. Qian, J. Prieto. **GENE THERAPY** 2000; 7:1824-1832.
102. "Expression of interferon-α subtypes in peripheral mononuclear cells from patients with chronic hepatitis C. a role for interferon-α5". E. Larrea, A. Alberdi, Y. Castelruiz, P. Boya, M.P. Civeira, J. Prieto. **JOURNAL OF VIRAL HEPATITIS** 2001; 8: 103-110.
103. "Gene Therapy of Orthotopic Hepatocellular Carcinoma in Rats Using Adenovirus Coding for interleukin-12 (IL-12)". M. Barajas, G. Mazzolini, G. Genové, R. Bilbao, I. Narvaiza, V. Schmitz, B. Sangro, I. Melero, C. Qian, J. Prieto **HEPATOLOGY** 2001; 33: 52-61.
104. "Genetic heterogeneity in the toxicity to systemic adenoviral gene transfer of interleukin-12". G. Mazzolini, I. Narvaiza, A. Pérez-Diez, C. Qian, B. Sangro, J. Ruiz, J. Prieto, I. Melero. **GENE THERAPY** 2001; 8: 259-267.
105. "IL-12 gene therapy for cancer: in synergy with other immunotherapies." I. Melero, G. Mazzolini, I. Narvaiza, C. Qian, L. Chen, and J. Prieto **TRENDS IN IMMUNOLOGY** 2001: 22: 113-115.
106. "Characterization of an immunologically conserved epitope from hepatitis C virus E2 glycoprotein recognized by HLA-A2 restricted cytotoxic T lymphocytes". P. Sarobe, E. Huarte, J.J. Lasarte, A.

López-Díaz de Cerio, N. García, F. Borrás-Cuesta, J. Prieto. **JOURNAL OF HEPATOLOGY** 2001; 34: 321-329.

107. Prognostic model for early acute rejection after liver transplantation" N. Gomez-Manero, JI. Herrero, J. Quiroga, B. Sangro, F. Pardo, JA. Cienfuegos, J. Prieto. **LIVER TRANSPLANTATION** 2001; 7: 246-254.
108. "Liver transplantation in cirrhotic patients with diabetes mellitus: Midterm results, survival, and adverse events". JJ. Blanco, JI. Herrero, J. Quiroga, B. Sangro, N. Gomez-Manero, F. Pardo, JA. Cienfuegos, J. Prieto. **LIVER TRANSPLANTATIO.N** 2001; 7:226-233.
109. "$\alpha_v\beta_3$ Integrin-mediated Adenoviral Transfer of Interleukin-12 at the Periphery of Hepatic Colon Cancer Metastases Induces VCAM-1 Expression and T-Cell Recruitment" G. Mazzolini, M. Barajas, C. Qian, J. Prieto, I. Melero. **MOLECULAR THERAPY**, 2001; 3: 665-672.
110. "Distributed Synthesis of Insulinlike Growth Factor I and its Binding Proteins May Influence Renal Function Changes in Liver Cirrhosis". C. Fernández-Rodríguez, I. Prada, A. Andrade, M. Moreiras, R. Guitián, R. Aller, J. Lledó, G. Cacho, J. Quiroga, J. Prieto. **DIGESTIVE DISEASES AND SCIENCES**, 2001; 46: 1313-1320.
111. "Antifibrogenic effect in vivo of low doses of insulin-like growth factor-I in cirrhotic rats". B. Muguerza, I. Castilla-Cortazar, M. García, J. Quiroga, S. Santidrian, J. Prieto. **BIOCHIMICA ET BIOPHYSICA ACTA**, 2001; 1536: 185-195.
112. "Gene Therapy of hepatocellular carcinoma with adenovirur expressing CD40L". V. Schmitz, M.A. Barajas, Y. Sun, J. Prieto, C. Qian. **HEPATOLOGY**, 2001; 34: 72-81
113. "Thrombopenic purpura induced by a monoclonal antibody directed to a 35-kilodalton surface protein (p35) expressed on murine platelets and endothelial cells". M. Rodríguez-Calvillo, I. Gabari, M. Duarte, G. Mazzolini, J. Rifón, E. Rocha, J. Prieto, I. Melero. **EXPERIMENTAL HEMATOLOGY**, 2001, 29: 589-595.
114. "Influence of Tumor Characteristics on the Outcome of Liver Tranasplantation Among Patients With Liver Cirrhosis and Hepatocellular Carcinoma". J.I. Herrero, B. Sangro, J. Quiroga, F. Pardo, M. Herraiz, J. A. Cienfuegos, J. Prieto. **LIVER TRANSPLANTATION**, 2001, 7: 631-636.
115. "Ductular morphogenesis and functional polarization of normal human biliary epithelial cells in three-dimensional culture". Y. Ishida, S. Smith, L. Wallace, T. Sadamoto, M. Okamoto, M. Auth, M.

Strazzabosco, L. Fabris, J. Medina, J. Prieto, A. Strain, J. Neuberger, R. Joplin. **JOURNAL OF HEPATOLOGY**, 2001, 35: 2-9.

116. "T-helper cell response to woodchuck hepatitis virus antigens after therapeutic vaccination of chronically-infected animals treated with lamivudine". S. Hervás-Stubbs, J. J. Lasarte, P. Sarobe, I. Vivas, L. Condreay, J. M. Cullen, J. Prieto, F. Borrás-Cueesta. **JOURNAL OF HEPATOLOGY**, 2001, 35: 105-111.
117. "Idiopathic Adulthood Ductopenia Long Term Follow-up After Liver Transplantation". R. Ríos, J.I. Herrero, J. Quiroga, B. Sangro, I. Sola, F. Pardo, J. A. Cienfuegos, M. Herraiz, J. Prieto. **DIGESTIVE DISEASES AND SCIENCES**, 2001, 46: 1420-1423.
118. "Protection against Woodchuck Hepatitis Virus (WHV) Infection by Gene Gun Coimmunization with WHV Core and Interleukin-12". R. García-Navarro, B. Blanco-Urgoiti, P. Berraondo, R. Sánchez de la Rosa, A. Vales, S. Hervás-Stubbs, J. J. Lasarte, F. Borrás, J. Ruiz and J. Prieto. **JOURNAL OF VIROLOGY**, 2001, 75: 9068-9076.
119. "Insulin-like growth factor-I restores the reduced somatostatinergic tone controlling growth hormone secretion in cirrhotic rats". I. Castilla-Cortázar, M. A. Aliaga-Montilla, j. Salvador, M. García, G. Delgado, S. González-Barón, J. Quiroga, J. Prieto. **LIVER**, 2001;21:405-9
120. "Nuclear Factor – kB in the Liver of Patients with Chronic Hepatitis C: Decreased RelA Expression Is Associated with Enhaced Fibrosis Progression". P. Boya, E. Larrea, I. Sola, P. Lorenzo Majano, C. Jiménez, M.P. Civeira, J. Prieto. **HEPATOLOGY**, 2001; 34: 1041-1048.
121. "Immune response in hepatitis C virus infection" P. Sarobe, J. J. Lasarte, A. López Díaz de Cerio, N. Casares, E. Huarte, L. Arribillaga, F. Borrás-Cuesta, I. Melero, J. Prieto. **INMUNOLOGÍA**; 2002, 20: 88-95.
122. "Tracing Transgene Expression in Cancer Gene Therapy: a Requirement for Rational Progress in the Field". B. Sangro, C. Qian, J. Ruiz, J. Prieto. **MOLECULAR IMAGING AND BIOLOGY,** 2002, 4, Nº 1: 27-33.
123. "Effect of ursodeoxycholic acid on methionine adenosyltransferase activity and hepatic glutathione metabolism in rats." C. M. Rodriguez-Ortigosa, R. N. Cincu, S. Sanz, F. Ruiz, J. Quiroga and J. Prieto. **GUT**, 2002, 50: 701-706

124. "Altered liver gene expressión in CCl_4-cirrhotic rats is partially normalized by insulin-like growth factor I". E. Mirpuri, E. R. García-Trevijano, I. Castilla-Cortazar, C. Berasain, J. Quiroga, C. Rodríguez-Ortigosa, J. M. Mato, J. Prieto, M. A. Ávila. **THE INTERNATIONAL JOURNAL OF BIOCHEMISTRY & CELL BIOLOGY**, 2002, 34: 242-252.
125. "Abnormal priming of CD4+ T-cells by dendritic cells expressing Hepatitis C Virus core and E1 proteins". Pablo Sarobe, Juan José Lasarte, Noelia Casares, Ascensión López-Díaz de Cerio, Elena Baixeras, Pablo Labarga, Nicolás García, FranciscoBorrás-Cuesta and Jesús Prieto. **JOURNAL OF VIROLOGY**, 2002;76:5062-70.
126. "An anti-ICAM-2 (CD102) monoclonal antibody induces immune-mediated regressions of transplanted ICAM-2-negative colon carcinomas." I. Melero, I. Gabari, A.L. Corbí, M. Relloso, G. Mazzolini, V. Schmitz, M. Rodriguez-Calvillo, I. Tirapu, E. Camafeita, J. P. Albar, J. Prieto. **CANCER RESEARCH,** 2002 , 62: 3167-3174.
127. "Identification of HLA-B27-Restricted cytotoxic T lymphocyte epitope from carcinoembryonic antigen" E. Huarte, P. Sarobe, J. J. Lasarte, G. Brem, E. H. Weiss, J. Prieto and F. Borras-Cuesta. **INT. J. CANCER**, 2002, 97: 58-63.
128. "Defective regulation of cholangiocyte Cl-/HCO3- and Na+/H+ exchangers in primary biliary cirrhosis". S. Melero, C. Spirlì, A. Zsembery, JF Medina, RE Joplin, E. Duner, M. Zuin, JM Neuberger, J. Prieto, M. Strazzabosco. **HEPATOLOGY** 2002; 35:1513-1521.
129. "Cytokine gene transfer into dendritic cells for cancer treatment" I. Tirapu, M. Rodriguez-Calvillo, C. Qian, M. Duarte, C. Smerdou, B. Palencia, G. Mazzolini, J. Prieto, I. Melero. **CURRENT GENE THERAPY,** 2002; 2:79-89
130. "Gene therapy for liver diseases: recent strategies for treatment of viral hepatitis and liver malignancies". V. Schmitz, C. Qian, J. Ruiz, B. Sangro, I. Melero, G. Mazzolini, I. Narvaiza, J. Prieto. **GUT** 2002, 50: 130-135.
131. "New strategies to enhance gene therapy efficiency" C Qian, B Sangro, J Prieto. **GASTROENTEROLOGY** 2002;123:639-42.
132. "Immunogene therapy of hepatocellular carcinoma and gastrointestinal tumors" J Prieto, B Sangro, I Melero, M Herraiz, G Mazzolini, I Narvaiza, M Barajas, C Qian. In "Immunology and the

Liver" Ed. Moreno-Otero R, Albillos, A, Garcia-Monzon, C. Accion Médica. Madrid. 2002 pag267-271

133. "A novel strategy for the generation of angiostatic kringle regions from a precursor derived from plasminogen". Schmitz, V. Prieto, J. Qian C.. **GENE THERAPY**,2002 ; 9:1600-6.
134. Efficacy and toxicity of intra-arterial cisplatin and etoposide for advanced hepatocellular carcinoma. Sangro B, Rios R, Bilbao I, Beloqui O, herrero JI, Quiroga J, Prieto J. **ONCOLOGY** 2002; 62:293-298
135. Gene Therapy of hepatocellular carcinoma and gastrointestinal tumors. Sangro B, Qian C, Schmitz V, Prieto J. **ANN N Y ACAD SCI** 2002; 963:6-12
136. Vaccination with an adenoviral vector encoding hepatitis C virus (HCV) NS3 protein protects against infection with HCV-recombinant vaccinia virus. Arribillaga L, de Cerio AL, Sarobe P, Casares N, Gorraiz M, Vales A, Bruna-Romero O, Borras-Cuesta F, Paranhos-Baccala G, Prieto J, Ruiz J, LasarteJJ. **VACCINE.** 2002 ;21:202-210.
137. Identification of an antigenic epitope for helper T lymphocytes from carcinoembryonic antigen.. Kobayashi H, Omiya R, Ruiz M, Huarte E, Sarobe P, Lasarte JJ, Herraiz M, Sangro B, Prieto J, Borras-Cuesta F, Celis E. **CLIN CANCER RES**. 2002 ; 8:3219-25
138. The woodchuck interferon-alpha system: Cloning, family description, and biologic activity. Berraondo P, Garcia-Navarro R, Gonzalez-Aseguinolaza G, Vales A, Blanco-Urgoiti B, Larrea E, Riezu-Boj JI, Prieto J, Ruiz J. **J MED VIROL**. 2002;68:424-32.
139. A recombinant adenovirus encoding hepatitis C virus core and E1 proteins protects mice against cytokine induced liver damage. Lasarte JJ, Sarobe P, Boya P, Casares N, Arribillaga L, López-Díaz de Cerio A, Gorraiz M, Borrás-Cuesta F and Prieto J. **HEPATOLOGY** 2003;37:461-470.
140. A Multidrug Resistance 3 Gene Mutation Causing Cholelithiasis, Cholestasis of Pregnancy and Adulthood Biliary Cirrhosis. Lucena JF, Herrero JI Quiroga, Sangro B, Herraiz M, Garcia-Foncillas J, Zabalegui N, Sola J, Medina, JF, Prieto J. **GASTROENTEROLOGY**, 2003;124:1037-1042
141. "In vitro and in vivo comparative study of chimeric liver-specific promoters". M.G. Kramer, M. Barajas, N. Razquin, P. Berraondo, M.

Rodrigo, C. Wu, C. Qian, P. Fortes, J. Prieto. **MOLECULAR THERAPY**, 2003;7:375-385.

142. "Liver failure caused by herpes-simplex virus thymidine-kinase plus ganciclovir therapy is associated with mitochondrial dysfunction and mitochondrial DNA depletion" MT. Herraiz, N. Beraza, A. Solano, B. Sangro, J. Montoya, J. Prieto, M. Bustos. **HUMAN GENE THERAPY**, 2003;14:463-472.
143. A synthetic peptide from transforming growth factor beta type III receptor, inhibits liver fibrogenesis in rats with carbon tetrachloride liver injury. Ezquerro I, Lasarte JJ, Dotor J, Castilla-Cortázar I, Bustos M, Peñuelas I, Blanco G, Rodríguez C, G Lechuga MC,. Greenwel P, Rojkind M, Prieto J, Borrás-Cuesta F. **CYTOKINE**, 2003 (in press)
144. Pancreatic cancer escape variants that evade immunogene therapy through loss of sensitivity to IFN-g induced apoptosis. G. Mazzolini, I Narvaiza, LA Martinez-Cruz, A Arina, M Barajas, JC Galofré, C Qian, JM Mato, J Prieto and I Melero **GENE THERAPY** (in press)
145. Expression of Wilms' tumor suppressor in the cirrhotic liver: relationship to HNF4 levels and hepatocellular function. C Berasain, JI Herrero, E R García-Trevijano, MA Avila, JI Esteban, JM Mato and J Prieto. **HEPATOLOGY** (in press)
146. Protection against liver damage by cardiotrophin-1: a hepatocyte survival factor upregulated in the regenerating liver. M Bustos, N Beraza, JJ Lasarte, E Baixeras, P Alzuguren, T Bordet, J Prieto. **GASTROENTEROLOGY** 2003, 125: 192-201.
147. Engineering Th determinants for effcient priming of humoral and cytotoxic T cell responses. A Lopez-Diaz de Cerio, JJ Lasarte, N Casares, P Sarobe, M Ruiz, J Prieto, F Borras. **INT IMMUNOLOGY** 2003; 15:691-699
148. The promise of gene therapy in gastrointestinal and liver diseases.J Prieto, M Herraiz, B Sangro, C Qian, G Mazzolini, I Melero, J Ruiz **GUT** 2003, 52 (suppl II): ii49-ii54
149. Inhibiting the expression of specific genes in mammalian cells with 5' end-mutated U1 small nuclear RNAs targeted to terminal exons of pre-mRNA. P Fortes, Y Cuevas, F Guan, P Liu, S Pentlicky, SP Jung, ML Martinez-Chantar, J Prieto, D Rowe, S Gunderson. **CELL BIOLOGY** , (in press)
150. Suppression of angiogenesis and tumor growth by adenoviral-mediated gene transfer of pigment epithelium-derived factor. L Wang,

V Schimtz, A Perez-Mediavilla, I Izal, J Prieto, C Qian. **MOLECULAR THERAPY**, (in press).

151. Anticardiolipin antibodies in chronic viral hepatitis. Do they have clinical consequences?. J.R. Yuste, J Prieto. **EUROPEAN JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY** 2003, 15 (7): 717-719.
152. Prolonged and inducible transgene expression in the liver by a gutless adenovirus: a potential therapy for liver cancer. Lin Wang, Ruben Hernández-Alcoceba, Vijay Shankar S.G., Maider Zabala, Stefan Kochanek, Bruno Sangro, M. Gabriela Kramer, Jesus Prieto, and Cheng Qian. **GASTROENTEROLOGY** (in press).

A SELECTION OUT OF 60 ORIGINAL PAPERS IN SPANISH MEDICAL JOURNALS

1. Etiopatogenia de la colelitiasis. C. Gomez Lázaro, J. Prieto Valtueña. **REVISTA CLINICA ESPAÑOLA** 1973; Nº 4, 315-322.
2. Ultraestructura hepática en la litiasis biliar. **REVISTA CLINICA ESPAÑOLA** 1973; 129: 329.
3. Inducción enzimática hepática: I. Actividad enzimática en sujetos sometidos a ingesta crónica de inductores. II Respuesta a la inducción en enfermos hepatobiliares. J. González Macías, J. Prieto Valtueña y S. de Castro del Pozo. **MEDICINA CLINICA** 1975; 64: 342.
4. Asociación de fibrosis hepática congénita y enfermedad de Caroli con expresión clínica secuencial de ambas anomalías. J.M. Prieto Valtueña, E. Señaris Rodríguez, M. Noya García, J.L. Vázquez Iglesias, J.J. Vidal Carreira y J. Potel Lesquereux. **GASTRO- ENTEROLOGIA Y HEPATOLOGIA**. 1979; 2: 245-249.
5. "Síndrome de Budd-Chiari asociado a hemoglobinuria paroxística nocturna". S. Pérez Pombo, A. Alvarez Prechous, J. Castillo, M. Otero Echart, P. Diéguez y J.M. Prieto Valtueña. **GASTROENTEROLOGIA Y HEPATOLOGIA** 1981; 4 (1): 47-51
6. "Autoanticuerpos circulantes en la cirrosis hepática criptogenética. Lesiones pulmonares y renales asociadas". M.P. Civeira, E. Ortiz de Landázuri, E. Cuadrado y J. Prieto. **GASTROENTEROLOGIA Y HEPATOLOGIA** 1982; 5 (6): 327-332.

7. "Esteatohepatitis no alcohólica" S. Rull, J. Saavedra Belmonte, T. Tuñón, R. Ruiz Capellán y J. Prieto Valtueña. **GASTROENTEROLOGIA Y HEPATOLOGIA** 1982; 5 (10).
8. "Enfermedad pulmonar eosinófila por fasciola hepática. Descripción de un caso y revisión de la literatura". L. Aliaga, M. Díaz, J. Quiroga, J.M. Aréjola, J. Prieto. **MEDICINA CLINICA** 1984; 82 (17); 764-767.
9. "Tratamiento con ciclofosfamida de la tumefacción parotídea del síndrome de Sjogren". L. Aliaga, J. Quiroga y J. Prieto. **MEDICINA CLINICA** 1984; 30 (5).
10. "Tratamiento de la fascioliasis humana con bithionol". J.M. Aréjola, J.A. Guisantes, M. Rubio, V. Martínez de Artola, M. Muñoz, F. Conchillo y J. Prieto. **GASTROENTEROLOGIA Y HEPATOLOGIA.** 1985; 8 (1).
11. "Lesión hepática aguda por inyección intraportal de E. Coli en conejos sensibilizados". M. Serrano Martínez, J.M. Lera Tricas y J. Prieto Valtueña. **REVISTA ESPAÑOLA DE LAS ENFERMEDADES DEL APARATO DIGESTIVO** 1985; 67 (2): 129-133.
12. "Respuesta renal a la expansión de volumen intravascular en perros sometidos a hipertensión portal post-sinusoidal aguda: Influencia de la síntesis renal de prostaglandinas". A. Mota, J. Quiroga, I. Colina, J.M. Zozaya, A. Chamorro, J. Díez, F. Guarner y J. Prieto. **NEFROLOGIA** 1985; 5 (4).
13. "Inhibición del sistema de cotransporte sodio, potasio en eritrocitos de pacientes con cirrosis hepatica". J.I. Varela, J. Díez y J. Prieto. **NEFROLOGIA** 1985; 5 (4).
14. "Contracturas en flexión asociadas a hipopituitarismo. Respuesta a la terapéutica con cortisol". J. Prieto, J. Suárez, J. Artieda y J.M. Aréjola. **MEDICINA CLINICA** 1986; 87 (3): 110-111.
15. "Peliosis hepática masiva después de ingesta prolongada de anticonceptivos orales". M. Serrano Martínez, C. Bernués Gambarte y J. Prieto Valtueña. **GASTROENTEROLOGIA Y HEPATOLOGIA** 1986; 9 (7): 344-347.
16. "Correlación entre los distintos marcadores de replicación del virus de la hepatitis B". J.I. Jaúregui, M. Serrano, M.P. Civeira y J. Prieto. **GASTROENTEROLOGIA Y HEPATOLOGIA** 1987; 10 (3): 105-109.
17. "Hemocromatosis idiopática. Revisión de la literatura y estudio de 17 pacientes". J. Suárez, L. Aliaga, L Sirvent, R. Nunes, O. Beloqui y J.

Prieto. **REVISTA DE MEDICINA DE LA UNIVERSIDAD DE NAVARRA** 1987; 31 (1): 15-21.

18."Actinomicosis primaria hepática". J. Suárez, J.M. Aréjola, R. Calderón, J. Prieto, A. Gómez, G. Zornoza. **REVISTA DE MEDICINA DE LA UNIVERSIDAD DE NAVARRA** 1987; 31 (1): 31-35.

19."Prevalencia de anticuerpos frente al virus herpes humano 6 (VHH-6 o HBLV) en la población general". M.P. Civeira, I. Cuende, A. Castilla y J. Prieto. **MEDICINA CLINICA** 1989; 92 (5): 199.

20."Déficit de alfa-1-antripsina. Alteraciones hepáticas asociadas a fenotipos diferentes de ZZ". L. Aliaga, M.A. Sauras, J. Fernández y J. Prieto. **GASTROENTEROLOGIA Y HEPATOLOGIA** 1989; 12 (2): 54-57.

21."Crioglobulinemia mixta y hepatitis crónica no A no B". A. Castilla Cortázar, J.I. Cuende Melero, J. Camps Bansell, B. Sangro Gómez-Acebo, F.J. Indart Urroz, O. Beloqui Ruiz y J. Prieto Valtueña. **REVISTA CLINICA ESPAÑOLA** 1989; 184 (7): 390-391.

22."Producción de citocinas y función monocitaria en la hepatitis crónica no A no B: relación con la actividad de la enfermedad". A. Castilla, S. Morte, M.P. Civeira, M.L. Subirá, M. Serrano y J. Prieto. **GASTROENTEROLOGIA Y HEPATOLOGIA** 1989; 12 (9): 462-467.

23."Tratamiento de la hepatitis crónica B con Interferón Alfa Linfoblastoide". A. Castilla, M.P. Civeira, J.I. Jaúregui, F. Pons, M. Serrano, S. Morte y J. Prieto. **MEDICINA CLINICA (BARCELONA)** 1989; 93: 601-603.

24."Virus herpes humano tipo 6 y síndrome de astenia crónica". J.I. Cuende, M.P. Civeira, J.I. Riezu-Boj, A. Castilla y J. Prieto. **MEDICINA CLINICA (BARCELONA)** 1990; 94: 721-724.

25."Regresión tumoral espontánea. A propósito de dos casos". M. Sureda, M.L. Subirá, S. Martín Algarra, J. Prieto Valtueña y B. Sangro. **MEDICINA CLINICA (BARCELONA)** 1990; 95: 306-308.

26."Síndrome de astenia crónica e infecciones víricas". J.I. Cuende, M. Serrano, J. Prieto. **REVISTA CLINICA ESPAÑOLA** 1991; 188 (5): 257-262.

27."Tratamiento de la cirrosis biliar primaria con ácido ursodesoxicólico. Resultados a corto y medio plazo y relación con el estudio de la enfermedad". L. García Villarreal, J.M. Zozaya, E. Macías, N. García González, J. Quiroga, F. Conchillo y J. Prieto. **REVISTA ESPAÑOLA DE LAS ENFERMEDADES DEL APARATO DIGESTIVO** 1991; 80 (5): 311-315.

28."Detección del DNA del virus de la hepatitis B en suero mediante amplificación génica en pacientes con hepatitis crónica B y en pacientes con hepatitis crónica C". J.I. Jaúregui, M.P. Civeira, M. Serrano, J. Camps, A. Castilla, J.I. Riezu-Boj y J. Prieto. **MEDICINA CLINICA (BARCELONA)** 1992; 98: 49-52.

29."Prótesis intrahepática portosistémica: PIP ("TIPS") en el tratamiento de la ascitis refractaria. Estudio piloto". L. García Villarreal, J.M. Zozaya Urmeneta, J. Quiroga Vila, B. Sangro Gómez-Acebo, J.I. Bilbao Jaureguizar, J. Longo Areso y J. Prieto Valtueña. **GASTROENTEROLOGIA Y HEPATOLOGIA** 1993; 16 (1): 8-12.

30."Actitud terapéutica en la hepatitis crónica". J.I. Herrero Santos y J. Prieto Valtueña. **GASTROENTEROLOGIA Y HEPATO-LOGIA** 1994; 17 (2): 96-104.

31."Hepatitis postransfusional en Navarra. Evidencia de infección aguda por el virus de la hepatitis C sin elevación de aminotransferasas". M.P. Huarte, M.D. Maluenda, M.P. Civeira, A. Medarde, J.M. Prieto. **MEDICINA CLINICA (BARC)** 1994; 103: 601-605.

32."Hemorragia digestiva alta en paciente con neurofibromatosis de von Recklinghausen". J.R. Yuste, O. Beloqui, A. de la Peña, I. Bilbao, N. García, J. Prieto. **REVISTA DE MEDICINA UNIVERSIDAD DE NAVARRA** 1995; 39: 8-11.

33."La paniculitis mesentérica en el diagnóstico diferencial del dolor abdominal". A. de la Peña Fernández, J.R. Yuste Ara, O. Beloqui Ruiz, J. Prieto Valtueña. **REVISTA ESPAÑOLA DE ENFERMEDADES DIGESTIVAS** 1996; 88 (7): 505-508.

34."Trasplante hepático en pacientes cirróticos mayores de 60 años". A. de la Peña, J.I. Herrero, B. Sangro, F. Pardo, J.L. Hernández, J. Alvarez-Cienfuegos, J. Quiroga, J. Prieto. **REVISTA ESPAÑOLA DE ENFERMEDADES DIGESTIVAS** 1998; 90 (1): 3-8.

35."Liver transplantation in cirrhotic patients over 60 years of age". A. de la Peña, J.I. Herrero, B. Sangro, F. Pardo, J.L. Hernández, J. Alvarez-Cienfuegos, J. Quiroga, J. Prieto. **REVISTA ESPAÑOLA DE ENFERMEDADES DIGESTIVAS** 1998; 90 (1): 9-14.

36."Linfoma difuso de células T de cérvix uterino: una localización inusual de un tumor poco frecuente". E. Pomares Arias, M. Payeras Mas, M.A. Conchillo Armendáriz, N. García Gonzalez, J. Prieto Valtueña. **ANALES DE MEDICINA INTERNA (Madrid)** 2000; 17 (8): 432-433.

37. "Terapia génica de las enfermedades hepáticas". B. Sangro, J. Ruiz, C. Qian y J. Prieto. **GASTROENTEROLOGÍA Y HEPATOLOGÍA (Barcelona)** 2000; 23 (8): 394-402.

38. "La terapia génica: sus aportaciones a las enfermedades hepáticas y renales". G. Mazzolini, J. Ruiz, C. Qian, J. Prieto. **NEFROLOGIA** 2001; Vol. XXI, Supl. 1, Pag. 17-23.

39. "Estrategias de terapia génica aplicadas al tratamiento de los tumores hepáticos". G. Mazzolini, J. Prieto. **ONCOLOGIA** 2001; Vol. 24 (6): 294-302.

40. "Fibrosis meidastínica idiopática asociada a hipercoagulabilidad. Apropósito de un caso". A. Bauzá Alonso, J. P. García Gómez, C. Abades Vázquez, N. García González, J. Prieto Valtueña. **ANALES DE MEDICINA INTERNA** 2001; VOL 18-Nº 12 638-640.

BOOKS

- **"TEMAS DE HEPATOLOGÍA"**. Garpyo Editorial. Madrid 1978.
- **"INTRODUCCIÓN A LA MEDICINA"**. J. Prieto y M. Fuster. EUNSA, Pamplona 1980.
- **"HEPATOLOGÍA ACTUAL"**. Ed. GARPYO, 1987. Junto con el Dr.Hernández Guío and other colaboraters.
- **"SERIE SALVAT DE CASOS CLÍNICOS"**. Directors: J. Rodés, J. Prieto, A. Rapado. Salvat Editorial, Barcelona 1991.
- **"ACTUALIDADES TERAPÉUTICAS EN LAS ENFERMEDADES HEPATOBILIARES"**. J. Rodés, J. Escartín, J. Guardia, J.M. Pajares, J.Prieto. Garsi Editorial. Madrid 1992.
- **"HEPATOBILIARY DISEASES"**. Eds. J. Prieto, J. Rodés, D.A. Shafritz. Springer-Verlag. Heidelberg (Alemania). October 1992.
- **"EL SISTEMA DEL INTERFERÓN Y LAS HEPATITIS VIRALES"**. J. Prieto, M.P. Civeira. Ene Editions. Madrid. 1993.

RESEARCH PROJECTS OF THE LAST 5 YEARS

1994:-1996 "Development of new strategies to treat Chronic Hepatitis B:Use of *marmota monax* as a animal model" DGCICY, PB93-1227

1995-1997: "Gene Therapy of Hepatocellular Carcinoma using Suicide Genes" (Fundacion Echebano)

1999-2002: "Prevention and therapy of woodchuck hepatitis virus infection using immunization with defective recombinant adenoviruses and gene transfer by means of gene gun" CICYT. SAF 99-0084

1999-2001: Biological effects of Interferon alpha 5 : interaction with hepatitis C virus (Fundacion Echebano)

2000-2003: "Targeted vectors for cancer gene therapy: receptor and transcriptional targeting of retroviral, lentiviral, and adenoviral vectors". European Commission. QLK3-CT-1999-00364

2000-2002: "Gene Therapy of neoplastic and viral diseases of the liver" Fundación Ramon Areces

Interferon-alpha5, the interferon-alpha subtype produced in the liver, signals stronger and induces higher expression of interferon-inducible genes in hepatocytic cells than interferon-alpha2

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Abbreviations: IFN, interferon; IFNAR, interferon alpha receptor; STAT, signal transducer and activator of transcription; PKC-δ, protein kinase C delta; ISRE, IFN-stimulated response element; IRF, IFN-regulatory factor; 2'-5' OAS, 2'-5' oligoadenylate synthetase; mRNA, messenger RNA; HCV, hepatitis C virus; PCR, polymerase chain reaction; Jak, janus kinase; Tyk, tyrosine kinase; GADPH, glyceraldehyde-3phosphate-dehydrogenase.

ABSTRACT

Interferon (IFN)-α5 is the only IFN-α subtype expressed constitutively in the human liver suggesting a specific role of this subtype in liver cells. However, IFN-α2, and not IFN-α5, constitutes the basis of the currently used antiviral therapy of chronic viral hepatitis. IFN-α2-based therapies fail to control viral replication in a great proportion of patients making necessary the introduction of more effective treatments for viral hepatitis. In this work we have compared IFN-α2 and IFN-α5 with respect activation of cell signaling cascades and induction of antiviral genes in hepatocytic cells. We found that the Tyr^{701} phosphorylation of STAT1 at 15, 30 and 60 minutes after stimulation with IFN was two times stronger when cells were incubated with IFN-α5 than when using IFN-α2. Similarly tyrosine phosphorylation of the receptor-associated kinase Tyk-2 at 10 and 30 minutes after exposure to IFN was 4-8 times more intense when using IFN-α5 than when using IFN-α2. Moreover, Tyr^{705} phosphorylation of STAT3 was 1.5-2 times higher with IFN-α5 than with IFN-α2 at 1, 5, 15, 30 and 60 min after stimulation with IFN. In parallel to these findings, the mRNA levels of the antiviral IFN-inducible genes 2′-5′ oligoadenylate synthase, p56 and MxA at 14h after incubation with IFN were about two times higher with IFN-α5 than with IFN-α2. In conclusion, our data show that IFN-α5 produces stronger activation of cell signaling and more efficient induction of IFN-

HCV genotype 1 infection,[14] the most prevalent genotype in the population. On the other hand IFN-α2-based therapy of chronic hepatitis B induces sustained virological response in less than 40% of HBe positive patients and in less than 30% of HBe negative cases.[15] Therefore, new therapies are urgently needed for all forms of chronic viral hepatitis.

IFN-α5, being the IFN-α subtype expressed in the hepatic tissue, might represent an alternative therapeutic agent for viral infections affecting the liver. In the present paper we show that IFN-α5 induces stronger activation signals and higher expression of antiviral genes than IFN-α2 in hepatocytic cells, suggesting differences in the interaction with the liver cell receptor. Our data offer grounds for consideration of IFN-α5 as a possible alternative therapeutic agent for chronic viral hepatitis.

MATERIAL AND METHODS

Cell culture. HepG2 human hepatoma cells (ATCC HB-8065) were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, Gaithersburg, Maryland) supplemented with penicillin (0.6 μg/ml), streptomycin (60 μg/ml) glutamine (2mM) and 10% fetal calf serum.(FCS).

Stimulation of cells with IFN. IFN-α2b was obtained from Schering-Plough (Madrid, Spain). Recombinant IFN-α5 was kindly provided by Dr. Vytautas Naktinis (Biotechna, Vilnius, Lithuania). HepG2 cells were seeded at 200.000 /well in 6 well plates in DMEM 10% FCS. For signal transduction analyses, cells were serum-starved for 8 h prior to IFN exposure. IFNs were used at 5000 U/ml in the presence of 2% FCS for periods indicated in each experiment.

Antibodies. Anti-phospho-STAT1^{tyr701}, anti-phospho-STAT3^{tyr705}, anti-phospho-Tyk-2$^{tyr1054/1055}$ antibodies and anti-Rabbit IgG HRP-linked antibody were purchased from Cell Signaling Bio-lab (Beverly, MA.). Anti-STAT3 antibody was obtained from Upstate Biotechnology (Lake Placid, NY, USA). Anti-STAT1 antibody was from Santa Cruz Biotech, Inc. Anti-actin and anti-Tyk-2 antibodies were from Sigma-Aldrich (Steinheim, Germany) and Transduction Labs (Lexington, KY) respectively.

Western blotting . After trypsinization, cells were collected by centrifugation. Cell pellets were resuspended and lysed in sample buffer containing dithiothreitol. Samples (50 μg protein) were resolved in SDS-PAGE under reducing conditions. Proteins were transferred onto nitrocellulose membranes (Bio-Rad laboratories, Inc) and stained with Ponceau red solution (Sigma-Aldrich,

sensitive genes than IFN-α2 in hepatocytic cells. These results support the consideration of IFN-α5 as a candidate therapeutic agent for chronic viral hepatitis.

INTRODUCTION

Interferons (IFNs) are a group of cytokines with pleiotropic effects including inhibition of cellular proliferation, induction of differentiation, modulation of the immune response and activation of an antiviral status in the cell.[1,2] Human type I IFNs include a multigene family of different IFN-α subtypes and a single IFN-β. All type I IFNs are structurally related and share the same IFN receptor which is constituted at least by two subunits: IFNAR1 and the full-length IFNAR2c form.[1] The diverse activities of type I IFNs are mediated by conserved signal trasduction pathways.[3,4] Binding of IFNα/β to its receptor, triggers signals that are transmitted through signal transducers and activators of transcription (STATs) from cell surface receptor to the nucleus. Stimulation with IFNs-α/-β leads to tyrosine phosphorylation of the Jak-1 and Tyk-2 receptor-associated kinases. These two Janus kinases are responsible for the rapid activation of STATs. Indeed, Jak-1 phosphorylates Tyr^{701} in STAT1 and Tyr^{690} in STAT-2 which form an oligomeric complex called ISGF3 also containing a third protein p48, a DNA binding protein. Tyrosine phsophorylation of STAT1 and STAT2 in response to IFN α/β occurs in all non-transformed cells. Full activity of STAT1 (isoform STAT1α) requires phosphorylation on Ser^{727} probably via PKC-δ/p38 mitogen-activated protein kinase pathway.[5,6,7] Then, ISGF3 translocates to the nucleus and activates the transcription of genes containing IFN-stimulated response elements (ISREs). IFN α/β also promote the formation of STAT1 homodimers, which bind to IFNγ activation sequence (GAS).[8] Activation of Tyk-2 kinase, is essential to phosphorylate Tyr^{705} in STAT3 and soon this factor is phosphorylated in Ser^{727} to be fully activated.[9,10,11] Tyrosine phosphorylation of STAT3, STAT4, STAT5 and STAT6 by type I IFNs takes place in a cell-type-specific manner.[4] Translocation of these transcription factors to the nucleus culminates in the activation of IFN type I-sensitive genes.[4] Of these genes, some are associated with regulation of apoptosis (caspases, Fas, p53), others with cell cycle arrest (p21, IFN-regulatory factor [IRF]-1), others with antiviral response (MxA, 2'-5' Oligoadenylate Synthetase (2'-5' OAS) and p56 proteins) and others with immunoregulatory activities (IRF1, IRF3 and MHC class I).[2,12]

Recently we found that IFN-α5 is the only IFN-α subtype which is expressed constitutively in the liver and that the level of IFN-α5 mRNA is markedly decreased in the liver of patients with chronic hepatitis C virus (HCV) infection.[13] At present, IFN-α therapy of chronic hepatitis C is based on the use of IFN-α2. However this agent, even in the pegylated formulation and in association with ribavirin, fails to control viral replication in more than 50% of patients with

Steinheim, Germany) to verify equal loading of proteins. Membranes were incubated in TBS-T (50 mM Tris-HCl (pH 7.6) 200 mM NaCl, and 0,1% Tween-20) with 5 % dry milk. Proteins were detected by incubation with the specific antibody in TBS-T. After extensive washing in TBS-T, horseradish peroxydase-conjugated antibody was added for 1 h. Membranes were subjected to extensive washings in TBS-T and the specific protein bands were visualized using the enhanced chemiluminiscence detection system (Perkin Elmer, Boston, MA), according to manufacturer's instructions. For reprobing, blots were stripped from membranes following the instructions of manufacturer. Membranes were autoradiographed and bands were quantified by densitometric analysis performed by Molecular Analyst/PC software (Bio-Rad laboratories, Inc).

Analysis of mRNA expression by quantitative real-time PCR . Total RNA was extracted from HepG2 cells using Ultraspec Reagent (Biotex, Houston, TX). One microgram of RNA was treated with DNase (Gibco-BRL, Paisley, U.K.) prior to reverse transcription with M-MLV Reverse Transcriptase (Gibco-BRL) in the presence of RNaseOUT (Gibco-BRL). MxA, p56 and 2'-5' OAS expression was measured by quantitative real-time PCR using a LightCycler (Roche Diagnostic GmbH, Mannheim, Germany), and the LC-DNA Master SYBR Green mix. Aliquots of 3 µl from dilution 1/10 of the cDNA pool were used for each polymerase chain reaction (PCR), containing upstream and downstream primers specific for each gene (Table 1) in a 10 µl final volume. To determine the specificity, the PCR products were analyzed by melting curves. As an internal control for each sample, PCR amplification of a fragment of GADPH cDNA was performed. The amount of each transcript was expressed by the formula: $2^{cp(GADPH)-cp(gene)}$, being cp the point at which the fluorescence rises appreciably above the background fluorescence.

Statistical analysis

Normality was assessed with the Shapiro-Wilks test. Statistical analyses were performed using parametric (Student's *t*) and non-parametric (Kruskal-Wallis and Mann-Whitney *U*) tests. All *P*-values were two-tailed and considered being significant if the associated value was less than 0.05. Descriptive data for continuous variables are reported as means ± SD or as medians and interquartile range. SPSS 9.0 for Windows was used for the statistical analysis.

RESULTS

STAT1-phosphorylation by IFN-α5 and IFN-α2: a comparative study in HepG2 cells

We compared the ability of IFN-α5 and IFN-α2 to activate STAT1, a critical step to initiate IFN-stimulated effects.[16] HepG2 cells were starved in DMEM for 8 h prior to IFN exposure.

Following stimulation with IFN, cells were collected at different time points and cell lysates were analyzed by western blot with antibodies recognizing specifically STAT1 or the tyrosine phosphorylated form. Both IFN-α subtypes rapidly induced STAT1-tyr^{701}. However, IFN-α5 induced stronger STAT1-tyr^{701} signal than IFN-α2 at 15, 30 and 60 minutes after stimulation (Fig. 1A). The densitometric values of the STAT1-tyr^{701} band were about two times higher for IFN-α5 than for IFN-α2 at all these time points (Fig. 1B). Values at minutes 1 and 5 after stimulation with IFN were similar for the two IFN-α subtypes (data not shown).
No differences with respect STAT1-Ser^{727} phoshorylation were observed after incubation of the cells with IFN-α2 or IFN-α5 (data not shown).

IFN-α5 induces higher Tyk-2 phosphorylation than IFN-α2 in HepG2 cells

Activation of Tyk-2 kinase, is essential to phosphorylate Tyr^{705} in STAT3. We compared the kinetics of Tyk-2 tyrosine phosphorylation induced by IFN-α2 and IFN-α5 in HepG2 using an antibody that recognizes Tyk-2 phosphorylation in 1054/1055 Tyr position. Induction of phospho-Tyk-2 from min 10 to min 60 following stimulation with IFN was more potent when cells were incubated with IFN-α5 than when using IFN-α2 (Fig.2A). Densitometric scanning showed that the p-Tyk-2 bands at min 10 , 30 and 60 were, respectively, 4, 8 and 1.5 times more intense with IFN-α5 than with IFN-α2 (Fig.2B).

IFN-α5 induces higher STAT3 tyrosine phosphorylation than IFN-α2 in HepG2.

STAT3 is associated with the IFNAR1 subunit and it is phosphorylated by Jak-1/Tyk-2 kinases when cells are treated with IFN type I.[3,9] We compared the ability of IFN-α5 and -α2 to activate this transcription factor in HepG2 cells by analyzing protein extracts from cells sampled before and at various time points after addition of IFN using specific antibodies for STAT3, STAT3-Tyr^{705} or actin. We performed experiments to determine the activation kinetics of STAT3-Tyr^{705} at early (1-30 min) and late (15-120 min) time points after stimulation with IFN (Figs 3A and 3B, respectively). IFN-α produced phosphorylation of STAT3 with a peak at 15 min either when using IFN-α2 or IFN-α5. However IFN-α5 induced stronger activation signal of STAT3 from min 1 to min 60. Densitometric values of the STAT3-Tyr^{705} band were 1.5-2 times higher with IFN-α5 than with IFN-α2 at 1, 5, 15, 30 and 60 min after stimulation with IFN (Fig. 3A and 3B).
With respect STAT3 phosphorylation in Ser^{727} we observed a signal of low intensity in cells previously to the addition of IFN. Stimulation with either IFN-α5 or IFN-α2 did not induce any substantial change in the intensity of the STAT3-Ser^{727} signal with respect total STAT-3 protein contained in lysates (not shown).

Comparative analysis on the expression of IFN-inducible genes by IFN-α5 and IFN-α2.

We next studied whether the differences found between IFN-α5 and IFN-α2 on their ability to activate STAT1 and Tyk-2/STAT3 signaling pathways was paralleled, or not, by differences in their power to stimulate the expression of antiviral IFN-sensitive genes. Thus, we measured by real-time quantitative PCR the mRNA levels of 2′-5′ OAS, MxA, and p56 in HepG2 cells at 14h after stimulation with IFN-α2 or IFN-α5. As shown in figure 4 both IFN-α subtypes were able to increase significantly the steady-state levels of mRNA of the three genes analyzed. However, as compared with IFN-α2 , IFN-α5 induced significantly higher mRNA levels of the three genes , 2',5'-OAS ($p<0.001$), p56 ($p<0.001$) and MxA ($p<0.007$).

DISCUSSION

IFN-α constitutes not a single molecule but a family of cytokines composed of at least 13 subtypes[17] which show a close similarity at the structural level and exhibit an homology of 80-100 % in the aminoacid sequence.[18] All of them interact with the same receptor and induce similar biological effects including antiviral, antiproliferative and immunomodulatory activities.[1,12] The reason for the presence of so many IFN-α subtypes with highly structural homology among them remains obscure. However recent data suggest that the sites that bind to the receptor may differ among subtypes and that some subtypes would bind to the receptor with higher efficiency than others.[19,20] Moreover some reports have shown that the intensity of biological effects on specific cell types can be different for the diverse IFN-α subtypes.[21,22] Thus, it seems that small variations in the primary sequence of the ligand may influence its interaction with the receptor resulting in differences in the transmission of the signal.[23]

Examination of primary structure of IFN-α2 and IFN-α5 proteins reveals 85% of homology between them, with 24 differences in aminoacid residues. Notably, it has been shown that Arg at position 22 of IFN-α2 sequence is important with respect antiviral activity.[23] In IFN-α5, position 22 consists of Gly (a non charged residue) instead of Arg (a positively charged residue), a change that could influence the electrostatic interaction with the receptor and intracellular signaling. Moreover it has been proposed that residues 24 to 29 of the IFN-α2 sequence are involved in binding to the receptor.[23] The replacement of Leu (a hydrophobic residue) at position 26 of IFN-α2 by a Pro (a hydrophylic residue) in IFN-α5 may cause a change of the tridimensional structure of interferon that may modify the affinity for the receptor and the biological activity. Hence, we found reasonable to investigate whether IFN-α2 and IFN-α5 could exert different biological responses on hepatocytic cells.

The interest in comparing IFN-α2 and IFN-α5 with respect their effects on liver cells also stems from our previous finding that the only IFN-α subtype expressed constitutively in the liver is IFN-α5.[13] Interestingly the mRNA levels of IFN-α5 are markedly decreased in chronic hepatitis C[13] possibly reflecting a viral strategy to escape to the endogenous interferon system. Here we show that IFN-α5 induces more potent activation of STAT-1, Tyk-2 and STAT3 than IFN-α2. These differences in signaling are accompanied by a more intense induction of antiviral IFN-inducible genes. Thus the mRNA levels of p56, MxA and 2′-5′ OAS in HepG2 cells after stimulation with IFN-α5 are approximately two times higher than when cells were activated with IFN-α2. Although it has been shown that IFN-α8 is as potent as IFN-α5 (and more than IFN-α2) with respect protection against ECM virus in HuH7 cells,[22] IFN-α8, in contrast to IFN-α5, is not naturally expressed in the normal nor in the diseased liver. Since IFN-α5 is not available to treat liver patients it is not possible to compare *in vivo* its biological effects with those of other IFN-α subtypes in the whole ecosystem of the liver composed of a variety of cell types. However the fact that hepatic IFN-α expression is restricted to IFN-α5 suggests, together with our *in vitro* findings, that this particular subtype may display more efficient antiviral activities in the hepatic tissue than other subtypes including IFN-α2.

In conclusion, we show that IFN-α5 induces stronger intracellular signaling and higher expression of antiviral interferon-inducible genes than IFN-α2. Our data offer grounds for consideration of IFN-α5, the IFN-α subtype expressed constitutively in the liver, as a possible alternative antiviral therapy for patients with liver disease.

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REFERENCES

1. Pestka S, Langer JA, Zoon KC, Samuel CE. Interferons and their actions. Annu Rev Biochem. 1987;56:727-777.

2. Goodbourn S, Didcock L, Randall RE. Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. J Gen Virol. 2000;81:2341-2364.
3. Platanias L C, Fish EN. Signaling pathways a ctivated b y i nterferons. E xp H ematol. 1 999;27:1583-1592.
4. David M. Signal transduction by type I interferons. Biotechniques. 2002 Oct;Suppl:58-65.
5. Wen Z, Zhong Z, Darnell JE Jr. Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation. Cell. 1995;82:241-250.
6. Decker T, Kovarik P. Serine phosphorylation of STATs. Oncogene. 2000;19:2628-2637.
7. Uddin S, Sassano A, Deb DK, Verma A, Majchrzak B, Rahman A, Malik AB, et al. LC.Protein kinase C-delta (PKC-delta) is activated by type I interferons and mediates phosphorylation of Stat1 on serine 727. J Biol Chem. 2002;277:14408-14416.
8. Lew DJ, Decker T, Strehlow I, Darnell JE. Overlapping elements in the guanylate-binding protein gene promoter mediate transcriptional induction by alpha and gamma interferons. Mol Cell Biol. 1991;11:182-191.
9. Yang CH, Shi W, Basu L, Murti A, Constantinescu SN, Blatt L, Croze E, et al. Direct association of STAT3 with the IFNAR-1 chain of the human type I interferon receptor. J Biol Chem. 1996;271:8057-8061.
10. Rani MR, Leaman DW, Han Y, Leung S, Croze E, Fish EN, Wolfman A, et al. Catalytically active TYK2 is essential for interferon-beta-mediated phosphorylation of STAT3 and interferon-alpha receptor-1 (IFNAR-1) but not for activation of phosphoinositol -kinase. J Biol Chem. 1999;274:32507-32511
11. Su L, David M. Distinct mechanisms of STAT phosphorylation via the interferon-alpha/beta receptor.Selective inhibition of STAT3 and STAT5 by piceatannol. J Biol Chem. 2000;275:12661-12666.
12. Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. How cells respond to interferons. Annu Rev Biochem. 1998;67:227-264
13. Castelruiz Y, Larrea E, Boya P, Civeira MP, Prieto J. .Interferon alfa subtypes and levels of type I interferons in the liver and peripheral mononuclear cells in patients with chronic hepatitis C and controls. Hepatology. 1999;29:1900-1904.
14. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet. 2001;358:958-965.
15. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology. 2001;34:1225-1241.
16. Kisseleva T, Bhattacharya S, Braunstein J, Schindler CW.Signaling through the JAK/STAT pathway, recent advances and future challenges. Gene. 2002;28:1-24.
17. Diaz MO, Bohlander S, Allen G. Nomenclature of the human interferon genes. J Interferon Cytokine Res 1996;16:179-180.
18. Henco K, Brosius J, Fujisawa A, Fujusawa JI, Haynes JR, Hochstadt J, Kovacic T, et al. Structural relationship of human interferon alpha genes and pseudogenes. J Mol Biol. 1985;185:227-260.

19. Platanias LC, Uddin S, Colamonici OR. Tyrosine phosphorylation of the alpha and beta subunits of the type I interferon receptor.Interferon-beta selectively induces tyrosine phosphorylation of an alpha subunit-associated protein. J Biol Chem. 1994;269:17761-17764.
20. Pfeffer LM. Biologic activities of natural and synthetic type I interferons. Semin Oncol. 1997;24(Suppl 9):S9-63-S9-69.
21. Foster GR, Finter NB. Are all type I human interferons equivalent? J Viral Hepat. 1998;5:143-152.
22. Foster GR, Rodrigues O, Ghouze F, Schulte-Frohlinde E, Testa D, Liao MJ, Stark GR, et al. Different relative activities of human cell-derived interferon-alpha subtypes:IFN-alpha 8 has very high antiviral potency. J Interferon Cytokine Res. 1996;16:1027-1033.
23. Viscomi, G.C. Structure-activity of type I interferons. Biotherapy. 1997;10:59-86.

Table 1. Primer sequences used in the study

Gene	Upstream primer (5′-3′)	Downstream primer (5′-3′)
2′-5′ OAS	TTAAGAGGCAACTCCGATGG	AGCAGACTGCAAACTCACCA
P56	ACCTGGGGCAACTTTGCCTGG	CAAAGCAGGCCTTGGC
MxA	ATCGGGGACCAGAG	ATGTAGCCCTTCTTCAG
GAPDH	GGTCGGAGTCAACGGATTT	CCAGCATCGCCCCACTTGA

Legend to figures

Fig. 1 Differential induction of STAT1-tyrosine phosphorylation by IFNα-2 and IFN-α5 . ***A:***. Analysis of STAT1 tyr-phosphorylation. HepG2 were untreated (time 0) or stimulated with 5.000 units/ml IFN-α2 or IFN- α5 for indicated times. Immunoblot analysis of total cell lysates for each treatment was assessed with the anti-STAT1-Tyr^{701}- antibody. The membrane was stripped and presence of total STAT1 protein was determined by using an anti-STAT1 antibody. Corresponding samples were also examined for actin concentrations by using anti-actin antibody as protein loading control. ***B*:** Results are also expressed as the fold induction of the STAT1-tyr/STAT1 ratio for each sample compared with the ratio obtained under untreated conditions. Results are representative of three independent experiments.

Fig.2 Differential activation of Tyk-2 by IFN-α2 and IFN-α5. ***A*** HepG2 cells were starved for 8 h and then incubated in medium 2% FCS in the absence (time 0) or presence of 5000 units/ml IFN-α2 or IFN-α5 for the indicated times. An anti-Tyk-2 phospho-Tyr–specific antibody was used to determine the Tyk-2 phosphorylation state by using whole cell lysates. Membrane was sequentially stripped and reprobed with antibody against Tyk-2 protein. ***B***. The blots were subjected for densitometry and phsopho-Tyk-2/Tyk-2 ratio changes relative to untreated cells are expressed as fold induction. Results are representative of three independent experiments.

Fig. 3. Differential induction of STAT3-Tyrosine phosphorylation by IFN-α2 and IFN-α5. ***A Left panel*:** Kinetics of STAT3 tyr-phosphorylation after IFN-α2 or IFN-α5 stimulation at early time points. HepG2 cells were starved for 8 h and subsequently untreated (time 0) or treated with 5000 units/ml IFN-α2 or IFN-α5 for the times depicted in the Figure. Cell lysates were immunoblotted with phospho-Tyr-specific STAT3 antibody or anti-actin antibody as loading control. *Right panel:* results are also represented as fold induction of STAT1-Tyr/actin ratio relative to unstimulated samples. ***B.*** *Left panel*: Analysis of STAT-3 tyr-phosphorylation at later time points. HepG2 were untreated (time 0) or stimulated with IFN-α2 or IFN- α5 for indicated times. Immunoblot analysis of total cell lysates for each treatment was assessed with an anti-STAT3-Tyr^{705}- antibody. The membrane was stripped and presence of total STAT3 protein was determined by using an anti-STAT3 antibody. Likewise, presence of actin protein was assessed by using an anti-actin antibody as loading control. *Right panel*, Results are also expressed as fold induction of the STAT3-tyr/STAT3 ratio compared with the ratio obtained under untreated conditions. Results are representative of three independent experiments.

Fig. 4 Differential antiviral gene induction between IFN-α2 and IFN-α5. 2'-5' OAS, p56 and MxA mRNA expression by real-time PCR in HepG2 unstimulated and stimulated for 14 h with IFN-α2 or IFN-α5. Results of 3 independent experiments in triplicate are represented

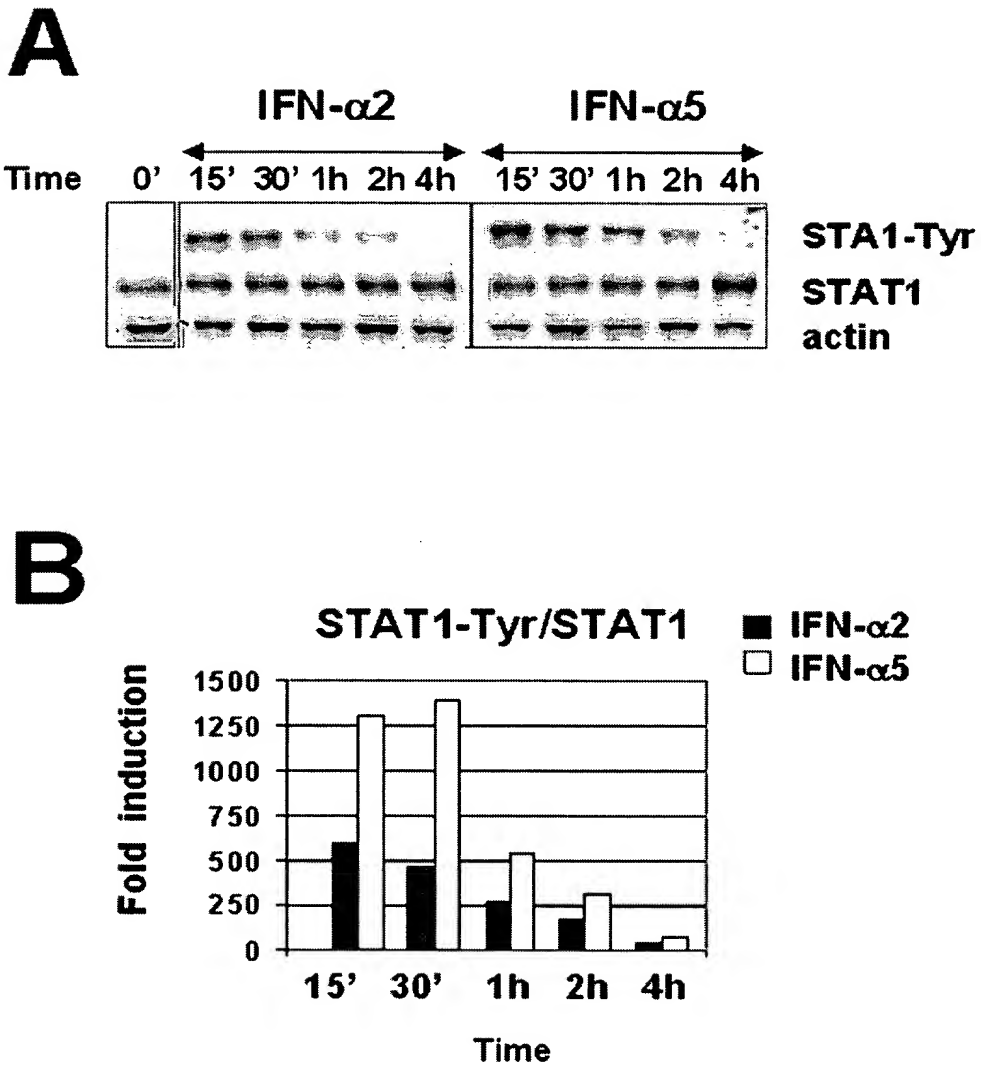
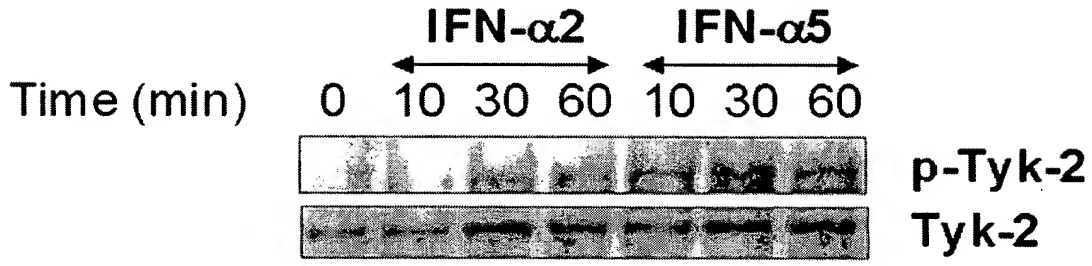

A
IFN-α2
IFN-α5
Time
0'
15'
30'
1h
2h
4h
15'
30'
1h
2h
4h
STA1-Tyr
STAT1
actin
B
STAT1-Tyr/STAT1
IFN-α2
IFN-α5
Fold induction
1500
1250
1000
750
500
250
0
15'
30'
1h
2h
4h
Time

Figure 1

A


IFN-α2
IFN-α5
Time (min)
0 10 30 60 10 30 60
p-Tyk-2
Tyk-2

B

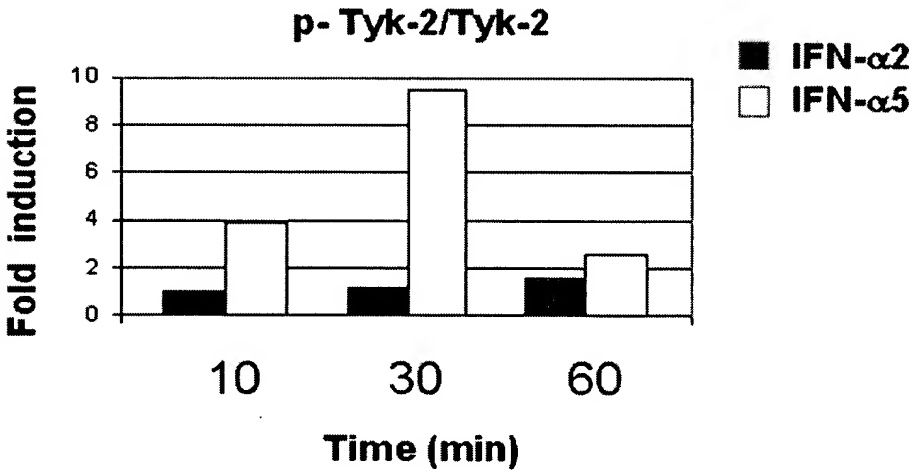

p- Tyk-2/Tyk-2
IFN-α2
IFN-α5
Fold induction
10
8
6
4
2
0
10
30
60
Time (min)

Figure 2

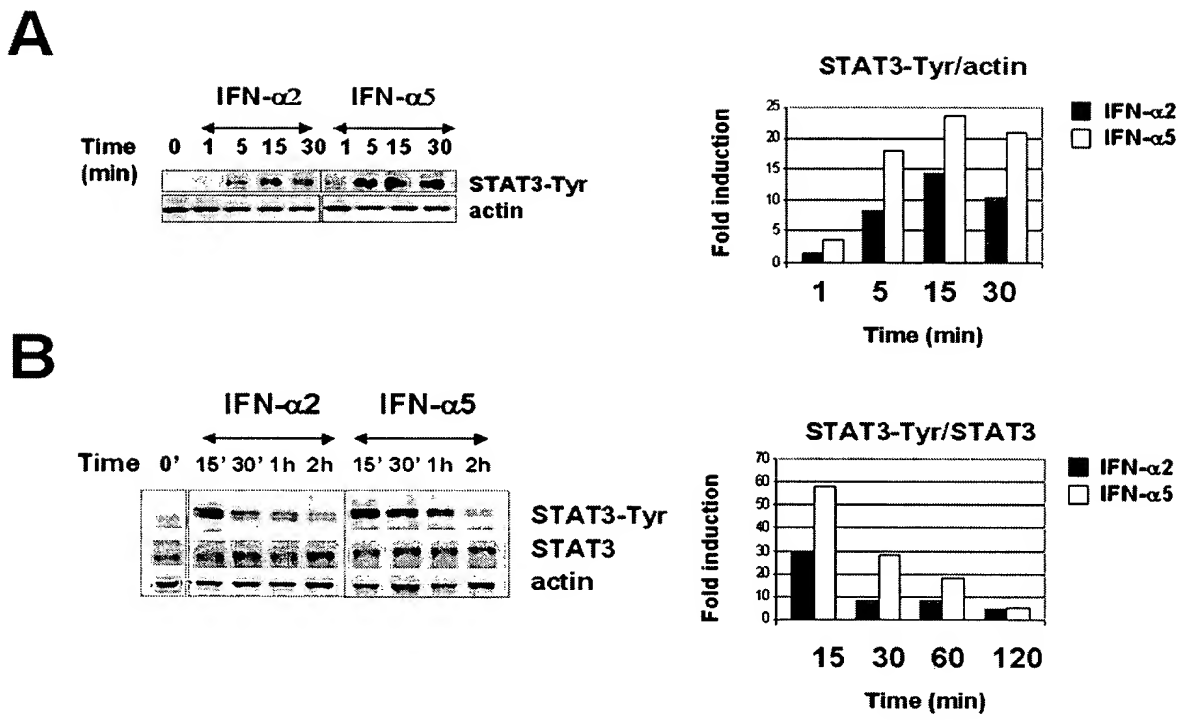

A
IFN-α2
IFN-α5
Time (min)
0 1 5 15 30 1 5 15 30
STAT3-Tyr
actin
STAT3-Tyr/actin
Fold induction
IFN-α2
IFN-α5
1 5 15 30
Time (min)
B
IFN-α2
IFN-α5
Time 0' 15' 30' 1h 2h 15' 30' 1h 2h
STAT3-Tyr
STAT3
actin
STAT3-Tyr/STAT3
Fold induction
IFN-α2
IFN-α5
15 30 60 120
Time (min)

Figure 3

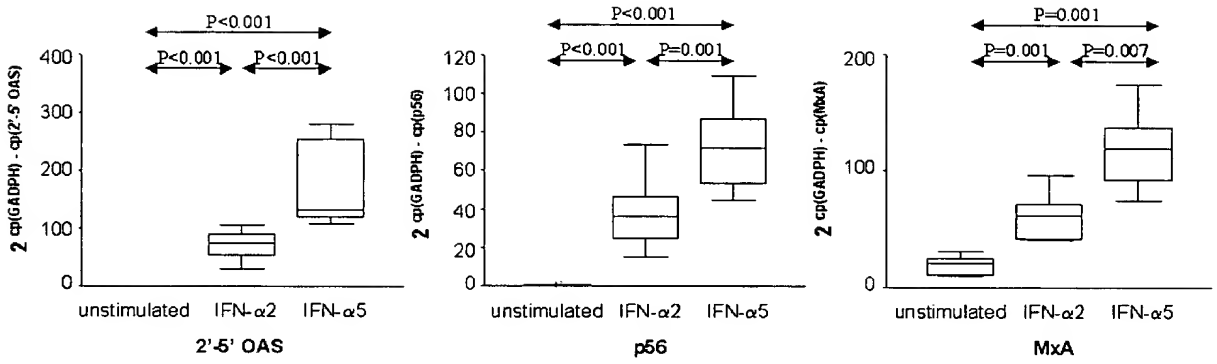

P<0.001
P<0.001
P<0.001
2 cp(GADPH) - cp(2'-5' OAS)
400
300
200
100
0
unstimulated
IFN-α2
IFN-α5
2'-5' OAS
P<0.001
P<0.001
P=0.001
2 cp(GADPH) - cp(p56)
120
100
80
60
40
20
0
unstimulated
IFN-α2
IFN-α5
p56
P=0.001
P=0.001
P=0.007
2 cp(GADPH) - cp(MxA)
200
100
0
unstimulated
IFN-α2
IFN-α5
MxA

Figure 4